Evolution of Uniparental Genetic Systems in Dermanyssine Mites (Acari: Mesostigmata)

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF LONDON

by

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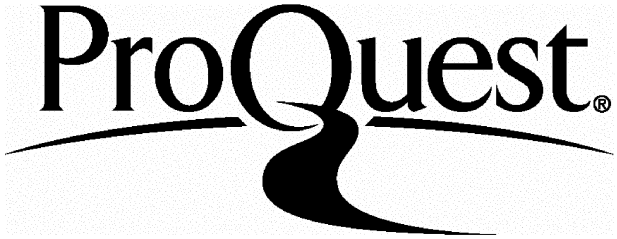

DEDICATION

This thesis is dedicated, with love, to my wife Alice who I met and married whilst working on this project and who has put up with so much to help me get it finished.

"The meaning of this degree is that the recipient of instruction is examined for the last time in his life, and is pronounced completely full. After this, no new ideas can be imparted to him."

Stephen Leacock "Sunshine Sketches of a Little Town"

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ABSTRACT

An exhaustive survey of uniparental genetic systems (those in which at least some individuals only transmit the genome of one parent to their offspring) within the Metazoa is presented including a new scheme for the classification of such systems (chapter 1). This is followed by a critical review of 13 different hypotheses proposed to account for the evolution of these systems (chapter 2). The hypothesis of Schrader and Hughes-Schrader, originally proposed in 1931 (*Q. Rev. Biol.* **6**: 411-438), is then introduced. This states that arrhenotoky (in which males arise from unfertilised eggs whilst females arise from fertilised eggs) evolves via an intermediate pseudoarrhenotokous stage (in which although males are produced biparentally they eliminate the genome of paternal origin at some point prior to spermatogenesis) rather than directly from a zygogenetic ancestor. The remainder of this thesis is concerned with testing this hypothesis. Criticisms of the hypothesis of Schrader and Hughes-Schrader made by Hartl and Brown in 1970 (*Theor. Popul. Biol.* **1**: 165-190) are re-examined in the light of more recent evidence and found to be invalid. Further evidence is cited from the recent literature in favour of the hypothesis of Schrader and Hughes-Schrader. A comprehensive review of the genetic systems of the Acari (mites and ticks) is used to ask whether our current state of knowledge in this field is sufficient to test the hypothesis of Schrader and Hughes-Schrader in this group. This reveals that the missing component required for such a test is a reliable hypothesis of phylogenetic relationships (chapter 3). A molecular phylogeny of the Dermanyssina (Acari: Mesostigmata) based on 755 base pairs of 28S ribosomal DNA is presented and this is shown to support the hypothesis of Schrader and Hughes-Schrader (chapters 4 and 5) A published morphological data set for the Dermanyssina is analysed and combined with the molecular data set in a variety of ways. An analysis of character conflict between the data sets is used to make an assessment of the suitability of the morphological characters for phylogenetic inference (chapter 6). Finally the hypothesis of Schrader and Hughes-Schrader is used to ask why is it always the males which are the haploid sex in haplodiploid species and ideas are presented for the future research (chapter 7).

→ p.22 paternal loss indicates
p.22 p.35 fertility!
p.25!
p.148 p.37

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Chapter One

Uniparental Genetic Systems and the Evolution of Haplodiploidy. I. Diversity of Uniparental Genetic Systems, Concepts and Definitions

"....our chauvinistic impression that haplodiploidy is rare should be revised. At least 10 percent of all named animal species are haplodiploid."

Stephen Jay Gould, 1983

SUMMARY

A uniparental genetic system is defined as any genetic system in which at least some individuals only transmit the genome from one parent to their offspring. This includes not only the familiar haplodiploid systems (arrhenotoky and pseudoarrhenotoky) but also a number of other theoretically plausible systems known from only one or a few examples or not recorded at all in nature. In this review an exhaustive survey is made of these genetic systems.

INTRODUCTION

The term haplodiploidy describes a phenomenon in which males are haploid whilst females are diploid. Haplodiploidy is frequent and widespread in nature and it has been estimated that over 10% of all named animal species are haplodiploid (Gould, 1983). It occurs not only in all Hymenoptera but also in all thrips (Thysanoptera) as well as some groups of nematode worms, rotifers, mites, beetles and scale insects. Haplodiploidy, however, represents only a restricted set of all the possible systems in which at least some individuals either arise uniparentally or fail to pass the genome of one of their parents to their offspring. I have called these uniparental genetic systems. Almost all secondarily derived uniparental genetic systems found in nature fall into one of two groups:

- **Maternal Daughter Systems** in which females give rise to females without the involvement of males. This is known as thelytoky or female parthenogenesis. Other maternal daughter systems are possible but these appear to be rare in nature.

- **Maternal Son Systems** in which males fail to pass a paternally derived genome to their offspring. Such systems include arrhenotoky in which males arise from unfertilised eggs whilst females arise from fertilised eggs, and pseudoarrhenotoky (also called parahaploidy or paternal genome loss (PGL) in which males receive a

paternal genome but this is lost at some point prior to spermatogenesis with the consequence that it is not passed on to the offspring.

There are also many other uniparental genetic systems which are logically possible but which do not seem to be represented in nature. All of these potential systems are considered in this review. There are at least five possible reasons why some uniparental genetic systems appear to be much more common than others:

- Common systems have a greater selective advantage and therefore arise more frequently.
- Common systems suffer from fewer genetic and developmental constraints and therefore arise more frequently.
- Common systems are more likely to lead to adaptive radiation and therefore they represent larger numbers of species.
- Rare systems are unstable and likely to either quickly revert to their plesiotypic state or evolve into some other genetic system, i.e., they arise just as frequently but are subsequently lost more frequently.
- Apparently rare systems are in fact common but they have not been detected due to observational bias.

HISTORICAL PERSPECTIVE

In 1845 a Silesian bee keeper, Johannes Dzierzon, who was probably a personal acquaintance of Gregor Mendel (Stubbe, 1965), proposed that male honeybees (*Apis mellifera*) arise from unfertilised eggs whilst females arise from fertilised ones (arrhenotoky) (Dzierzon, 1845a; Dzierzon, 1845b). It was not until 60 years later however, that Newell was finally able to confirm this to the satisfaction of (almost) all (Newell, 1915). A supporter of Dzierzon, Baron von Berlepsch, had shown that when the seminal receptacles of the queen bee were removed or the sperm immobilised by freezing for 36 hours, the queen bees produced only drones (Farley, 1982). In 1855 von Siebold and Leuckart visited von Berlepsch's apiary to test Dzierzon's hypothesis.

> *"By then Leuckart had discovered the micropyle in the insect egg through which he assumed the sperm travelled. Both men realised that it might be possible to observe the sperm in this region and thus to ascertain whether only queen and worker eggs actually contained them. Initially both men failed to establish any legitimate proof, but then von Siebold hit upon the idea of gently crushing the eggs so that they would rupture at the end opposite the micropyle. When he did this, the egg contents flowed out through the ruptured opening, leaving an empty space at the micropyle end. Examining this open space, he found spermatic filaments in thirty of the fifty-two worker eggs examined, but in none of the thirty-seven drone eggs examined. Thus he concluded that eggs, "when they are laid without coming into contact with the male semen, develop into male bees, but, on the contrary, when they are fertilised by male semen, produce female bees." [von Siebold, 1857]"*
>
> *John Farley, 1982*

Landois (1867) and later Dickel (1898) disputed Dzierzon's interpretations and claimed that rather than being predetermined in the egg, sex was determined much later, in the larva, as a response to differential diets. von Siebold (1857; 1867) and Bessels (1868) however, sided with Dzierzon as did von Paulcke (1899) and Petrunkewitsch (1901) who showed that eggs which developed in drone cells (special cells in the comb in which the larvae of males develop) lacked sperm, whilst those in other cells had been fertilised. In 1915 Newell eliminated any remaining doubt about the validity of Dzierzon's theory by performing hybridisation experiments between bees of distinct phenotypic races[1].

Although arrhenotoky was by now fully established as the mode of inheritance in the honeybee, arguments about whether this was a characteristic of the Hymenoptera as a whole, or just a peculiarity of *Apis mellifera* persisted (Castle, 1904; Forel, 1874; Lubbock, 1894; Wheeler, 1903; Wheeler, 1904) until well into the third decade of this century (Sanderson, 1932), in part due to the confusion created by the discovery of thelytoky in some other members of the Hymenoptera (Jack, 1917; Reichenbach, 1902; Tanner, 1892).

In 1903 von Lenhossék suggested that the queen bee carries two kinds of eggs, male and female, and that only the female eggs are capable of being fertilised, indeed require fertilisation for development to begin, whereas male eggs develop parthenogenetically (von Lenhossék, 1903). Between 1905 and 1907 Hertwig proposed his hypothesis of karyoplasmic ratio to explain sex determination and applied it to haploid parthenogenesis. When eggs of a honeybee develop parthenogenetically, he suggested, a readjustment of nuclear volume occurs which leads to a karyoplasmic ratio (i.e. a ratio of nuclear volume to cytoplasmic volume) characteristic of maleness (Hertwig, 1905; Hertwig, 1907), however by 1912 he had abandoned this hypothesis. In 1913 Nachtsheim and Oehninger independently reported that in the honeybee the nuclear volume in the two sexes is more or less the same (Nachtsheim, 1913; Oehninger, 1913), this further counted against the karyoplasmic ratio hypothesis, however it still admitted the possibility that the cytoplasmic volume of cells differs between the sexes furnishing the basis for a difference in karyoplasmic ratio. In 1926 Schrader and Hughes-Schrader showed that in *Icerya purchasi*, a scale insect in which nuclear volume varies between the sexes, the cytoplasmic volume varies proportionately holding the karyoplasmic ratio constant (Schrader and Hughes-Schrader, 1926). Whilst this was still to be demonstrated in the honey bee it more or less laid to rest the theory of karyoplasmic ratio.

It was these same two authors who five years later suggested that arrhenotoky could arise through a succession of stages involving parahaploid systems as observed in many scale insects (Schrader and Hughes-Schrader, 1931). Hartl and Brown (1970) pointed out that if this were true then these intermediate parahaploid forms should also be found in other

[1] But see Sanderson (1932) for an alternative interpretation of these experiments.

haplodiploid groups. At that time they did not know of any and they took this to be evidence that parahaploidy was not a necessary intermediate step in the evolution of arrhenotoky. Since then parahaploidy has been shown to be the genetic system of a number of species of mite and one species of beetle, both closely related to arrhenotokes. This subsequent discovery vindicates the ideas of Schrader and Hughes-Schrader from this criticism at least, and it is possible that further study of other less well known haplodiploid taxa will lead to the discovery of more parahaploid remnants. The apparent absence of parahaploidy from the hymenoptera by no means precludes its previous existence. The evolutionary origins of the hymenoptera lie far back in the past and, for reasons discussed below, parahaploidy may be a far less stable genetic system than arrhenotoky.

THE CONCEPT OF THE GENETIC SYSTEM

The concept of the **genetic system** was first introduced by Cyril D. Darlington in his landmark book *"The Evolution of Genetic Systems"* (Darlington, 1939). In most higher organisms the function of propagation is coupled to the generation of novel variation (by the cycle of meiosis and syngamy) such that there is a trade-off between genetic constancy and variability. The processes which maintain this balance are referred to collectively as the genetic system (Carson, 1975; Carson, 1987; Grant, 1958; Mather, 1943; Stebbins, 1950). These processes include control of mutation rate, recombination rate, length of life cycle and relative duration of vegetative and reproductive phases, fertility, gamete and seed dispersion, chromosome number, ploidy, breeding system and a host of other factors (Korol, Preygel and Preygel, 1994).

THE CONCEPT OF THE UNIPARENTAL GENETIC SYSTEM

Genetic systems can be divided into two types, biparental and uniparental. Any genetic system in which all individuals are biparental and pass the genomes of both parents to all of their offspring will be referred to as a **biparental genetic system**. All individuals in a biparental genetic system arise by **zygogenesis** (= gamogony) (the formation of a new individual by the union of two gametes (syngamy)). Biparental genetic systems in which adults of both sexes are somatically diploid are called **diplodiploidy**.

Any genetic system in which the offspring of at least some individuals only transmit the genome from one parent to their offspring will be referred to as a **uniparental genetic system**. If these individuals only transmit the genome of one parent because they only have one parent this will be called **prefertilisation uniparentalism**. If they receive genomes from two parents but only transmit one of these to their offspring this will be called **postfertilisation uniparentalism**. The offspring which only transmit the genome from one of their parents to their offspring in a uniparental genetic system will be referred to as the **uniparental offspring**. The parent from which uniparental offspring transmit

their genomes to their offspring are called the **uniparental parents**. The offspring which transmit the genomes of both parents to their offspring are called the **biparental offspring** and the parents from which uniparental offspring do not transmit their genomes to their offspring are called the **biparental parents**. Biparental genetic systems are generally considered to be plesiotypic with respect to uniparental systems.

DETERMINANTS OF UNIPARENTAL GENETIC SYSTEMS

Uniparental genetic systems can be classified according to the **determinant of uniparentalism**. The determinant of a uniparental genetic system is the feature of the parents which determines whether they are to be the uniparental parent or the biparental parent, for example if the uniparental parents are defined by their sex then this is a sexually determined uniparental genetic system. Sex however is not the only possible determinant of uniparentalism. Uniparentalism may be determined by parental species in hybrid organisms. This phenomenon is called **hybridogenesis** (Schultz, 1969) and occurs in the stick insect *Bacillus rossius-grandii benazzii* (Mantovani and Scali, 1992), fish of the genus *Poeciliopsis* (Schultz, 1969) and the hybrid frog *Rana esculenta*.

Uniparental Genetic Systems Determined by Parental Species

Rana esculenta (the edible frog) is a hybrid between *Rana ridibunda* (the marsh frog) and *Rana lessonae* (the pool frog) (Berger *et al.*, 1988; Uzzell, 1978; Uzzell and Berger, 1975; Uzzell, Gunther and Berger, 1975; Uzzell, Gunther and Berger, 1977). In populations of *R. esculenta* which are sympatric with one of their parent species the two will mate with each other. In areas where *R. esculenta* and *R. lessonae* are found together when a female *R. esculenta* is crossed with a male *R. lessonae* then all of the *lessonae* chromosomes are eliminated in the oogonial cells of the *R. esculenta* female (Tunner and Heppich-Tunner, 1991) so that she only passes on the *ridibunda* genome to form a new *R. esculenta* individual when combined with the *lessonae* genome of the father. In the reciprocal cross *R. esculenta* males eliminate the *lessonae* genome in spermatogonial cells (Bucci *et al.*, 1990; Jablonka and Lamb, 1995; Vinogradov *et al.*, 1990). If *R. esculenta* and *R. ridibunda* occur together then it is the *ridibunda* genome which is eliminated by *R. esculenta* which suggests that this is a balanced system which has evolved in order for *R. esculenta* to reproduce itself (and cope with the genetic demands of hybridogenesis) rather than the outcome of an evolutionary arms race between the genomes of the two parent species. Matings between *R. esculenta* males and females are rare in populations which are sympatric with one of the parent species but if both parental species are absent then such matings do occur and either parental genome may be eliminated. Since adults only pass on the genome of one of their parents this is a uniparental genetic system but the uniparental parent is not distinguished by its sex but by its species. This species determined uniparentalism has been called **parental dominance**

(Heslop-Harrison, 1990) and relies on species-specific imprinting just as sex determined uniparentalism relies on sex specific imprinting.

Schmidt (1993) considers this system of hemiclonal hybridogenesis to be analogous to cyclical parthenogenesis[2]. According to Schmidt, in areas in which *R. esculenta* and *R. lessonae* are sympatric, *R. esculenta* x *R. lessonae* pairings represent the asexual phase of a cyclical parthenogen whilst *R. esculenta* x *R. esculenta* pairings, which result in *R. ridibunda* offspring due to the elimination of the *lessonae* genome in both *R. esculenta* parents (Binkert *et al.*, 1982; Hotz *et al.*, 1992), represent the sexual phase. Frogs cannot reproduce parthenogenetically due to genetic and developmental constraints (the chromosome which forms the poles of the first mitotic spindle is always contributed by the sperm (Wake and Roth, 1989), genomic imprinting (Hurst and Hamilton, 1992) and other factors (Kirkpatrick and Jenkins, 1989). Despite these constraints on parthenogenesis hemiclonal hybridogenesis has enabled *R. esculenta* to achieve the same advantages which they would receive from cyclic parthenogenesis; occasional outbreeding in an otherwise uniparental genetic system which has the favourable properties of both sexual and asexual reproduction but the costs of neither (Hastings, 1991; Hastings, 1992a; Hastings, 1992b; Hedrick and Whittam, 1989). Mate choice by a *R. esculenta* female allows a female to either continue the asexual phase by mating with a *R. lessonae* male or enter the sexual phase by mating with a *R. esculenta* male. This would allow her to control the relative amounts of sexual and asexual reproduction in order to achieve level of sexual reproduction most appropriate to the circumstances. Since female *R. esculenta* prefer *R. lessonae* males to those of *R. esculenta* (Abt and Reyer, 1993), the amount of time spent in the sexual phase must be low. This supports the theoretical observations (Charlesworth *et al.*, 1993a; Charlesworth *et al.*, 1993b; Kondrashov, 1994) that only a small amount of recombination is sufficient to prevent extinction due to Muller's Ratchet.

Uniparental Genetic Systems Determined by Parental Sex

The most familiar uniparental genetic systems are those which are determined by sex. Uniparental genetic systems in which the uniparental offspring are male are called **uniparental son systems** whereas those in which the uniparental offspring are female are called **uniparental daughter systems**. Uniparental genetic systems in which the uniparental parents are male are called **paternal systems** whereas those in which the uniparental parents are female are female are called **maternal systems**. In uniparental son systems the biparental offspring are female. In uniparental daughter systems the biparental offspring are male. In systems in which the uniparental offspring are of both sexes there are no biparental offspring. In maternal systems the biparental parents are male. In paternal systems the biparental parents are female. Uniparental genetic systems

[2] But see Milinski (1994) and Schmidt (1994).

in which the uniparental parent is the female are common in nature and are collectively known by the more familiar term **parthenogenesis**. Those in which it is the male which is the uniparental parent are much less common and are collectively known by the term **androgenesis**. Since in each of these two classes the uniparental offspring may be either male or female there are four basic uniparental genetic systems determined by the sex of one parent (table 1.1). There are also, potentially, four systems which are composites of these basic systems (table 2.1.).

TEMPORAL SCALE IN UNIPARENTAL GENETIC SYSTEMS

Uniparental genetic systems can be classified on the basis of the timing of uniparentalism. At one extreme the uniparental parent only passes on the genome of a single parent because it only ever had one parent. This is called **prefertilisation uniparentalism**. At the other extreme the genome of the biparental parent is eliminated just prior to gametogenesis. The intermediate part of this temporal spectrum consists of elimination of the genome of the biparental parent at some point in the life-cycle of the uniparental offspring between fertilisation and gametogenesis. Any uniparental genetic system in which genetic material is passed from the biparental parent to the uniparental offspring is termed **postfertilisation uniparentalism**. In these cases uniparentalism is achieved by elimination of the genome of the biparental parent. These temporal classifications apply to all uniparental genetic systems, not only those determined by parental sex. For example the species determined uniparental genetic system of *Rana esculenta* is a postfertilisation system since all individuals have two parents but in regions of sympatry with one of the parental species *Rana esculenta* only pass on the genome they received from one of their parents to their offspring. Names of postfertilisation systems are derived by adding the prefix 'pseudo' to the name of the prefertilisation system e.g. pseudoarrhenotoky etc.

Genome elimination is generally achieved by marking the portion of the genome to be eliminated by **heterochromatisation**[3], followed by elimination of this heterochromatin.

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[3] The categorisation of chromatin into euchromatin and heterochromatin was first proposed by Heitz (1928). Heterochromatin replicates later in the S phase of the cell cycle than euchromatin (Khosla and Chandra, 1996; Pardue and Hennig, 1990; Zuckerkandl and Hennig, 1995) and restricts the accessibility of transcription factors to the DNA (Gottschling, 1992) but once a factor has gained access to a regulatory site during the short S phase of the cell cycle at which heterochromatin is decondensed in order to allow entry of the replication machinery (Murray and Hunt, 1993), the active state can by propagated stably for many generations which may give rise to different subpopulations of cells (Aparicio and Gottschling, 1994). The epigenetic inheritance of silent states at mating-type loci and position effect variegation in *Drosophila* are both characterised by a clonally inherited pattern of gene expression with genes expressed in some cells but not in others. There are many examples of heterochromatisation. These include dosage compensation by X inactivation in placental mammals (Ballabio and Willard, 1992; Grant and Chapman, 1988; Lyon, 1992), position-effect variegation (PEV) in mice (Cattenach, 1974), *Drosophila* (Wallrath and Elgin, 1995), and yeast (Zakian, 1995a; Zakian, 1995b), inactivation of chicken erythrocyte nuclei (Aubert *et al.*, 1991; Ringertz and Bergman, 1985), silencing of mating-type loci in yeast (Aparicio, Billington and Gottschling, 1991), heterochromatisation of telomeres implicated in ageing, intercalary heterochromatin and heterochromatisation of tandemly repeated simple sequence satellite DNA (e.g. alpha-satellite DNA in humans) in the centromeres of mammalian chromosomes which appears to play a structural role by mediating the attachment of the kinetochore. These examples, however, only constitute uniparental genetic systems in cases in which heterochromatisation is followed by elimination and this elimination is determined according to the parental origin of the chromatin in question. The process of heterochromatisation appears to be mediated by alteration of the structure of the nucleosome. Heterochromatisation is associated with reduced histone acetylation (Braunstein *etal.*, 1993; O'Niell and Turner, 1995) and heterochromatin is

By subverting the normal process of chromatin condensation[4] which occurs during every cell cycle (Murray and Hunt, 1993) the uniparental parent may be able to cause the heterochromatisation and subsequent elimination of the genome of the biparental parent in its uniparental offspring allowing it to increase the representation of its genes in subsequent generations. There may be a long period of stable mitotic transmission of heterochromatin before elimination occurs. Elimination of the paternal genome is called **paternal genome loss (PGL)**. Elimination of the maternal genome is called **maternal genome loss (MGL)**.

Any candidate for a single-gene model of paternal genome loss must fulfil the following criteria:

- One of the two genomes inherited by a male must be eliminated prior to spermatogenesis.
- Only the paternally derived genome must be eliminated.
- The paternal genome must be eliminated in its entirety.
- Paternal genome elimination should only occur in males.
- The genome to be eliminated should be marked by heterochromatisation prior to elimination.

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characterised by enhanced methylation compared to euchromatin. For example, of the 61 CpG dinucleotides of one CpG island located near the promoter of the X linked PGK1 gene 60 are methylated at the cytosine residue on inactivated chromosomes whereas none are methylated on the active X and treatment with 5-azacytidine, which causes demethylation, can even reactivate previously inactivated chromosomes.

[4] Chromatin condensation occurs as a normal part of the cell cycle but the mechanisms of condensation are related to those of heterochromatisation. Chromatin condensation is associated with dephosphorylation of the linker histone H1 (Bradbury and Matthews, 1974; Churchill and Travers, 1991; Roth and Allis, 1992) During spermatogenesis of the sea urchin chromatin condensation is correlated with dephosphorylation of a sperm specific linker histone (Green and Poccia, 1985; Green *et al.*, 1995). Heterochromatisation, which can be seen as an extreme form of chromosome condensation, also appears to be associated with dephosphorylation of linker histones and the difference between chromatin condensation and heterochromatisation seems to lie in the nature of the histone protein involved. Inactivation of the entire erythrocyte nucleus of the chicken occurs by heterochromatisation. The normal linker histone of somatic cells, H1, is replaced by a special linker histone called H5 which accumulates, compacting nucleosomal arrays much more effectively and repressing transcription (Aubert *et al.*, 1991). H5 is more argenine rich than H1 and it is probably this argenine richness that strengthens its interaction with DNA and stabilises the chromatin structure (Aubert *et al.*, 1991). Just as normal chromatin condensation is associated with dephosphorylation of H1, heterochromatisation is associated with dephosphorylation of H5. This process can be reversed by rephosphorylation of H5 which will reactivate the chicken erythrocyte (Ringertz and Bergman, 1985). This means that the normal process of chromatin condensation could be subverted by one parent in order to cause heterochromatisation of the genome of the other parent in the offspring by altering the structure of the linker histone in the chromatin contributed by the other parent. A molecular analysis of linker histones in the chromatin contributed by each parent in postfertilisation uniparental genetic systems such as those found in mites and scale insects would be an appropriate test of this hypothesis. Specifically the genome which is heterochromatised (i.e. that of the biparental parent) would be expected to contain a linker histone protein which has a higher affinity for DNA than that of the uniparental parent, furthermore this difference would only be expected in the uniparental offspring (i.e. the expression of the aberrant linker histone should be sex specific). Since in this model the linker histones are different in the two sexes, they could represent the basis for genomic imprinting observed in these species. In some organisms, e.g. mammals, genomic imprinting is mediated by specific differences in methylation patterns between the active and inactive copies of the gene. Embryos of such organisms which lack a functional DNA methyltransferase fail to carry out genomic imprinting due to their lack of the ability to methylate DNA (Reik and Allen, 1994). *Drosophila* DNA, however, does not usually contain 5-methyl cytosine (Uriel-Shoval, J and and Razin, 1982) and in fact methylated genes have never been detected in any invertebrate (Latchman, 1995) (although methylation may be associated with polytenisation in *Diptera* (Eastman *et al.*, 1980)). It seems unlikely therefore that imprinting is due to methylation of DNA in these organisms and modification of the protein component of the chromatin rather than the DNA itself may be responsible. Imprinting due to different linker histones or differential modification of linker histones may be more widespread than postfertilisation uniparentalism, indeed it may be a prerequisite for the evolution of such systems. Ciliate protozoa are the model organisms for studies of chromatin condensation (Allis and Gorovsky, 1981; Gorovsky, 1986; Lin and Allis, 1991; Roth and Allis, 1988). For more information on the role of changes in chromatin structure of individual genes in mediating commitment to a particular differentiated state see Latchman (1995).

Examples of the elimination of heterochromatin can be found in a number of different taxa including ciliate protists[5], nematodes[6], copepods[7] and Diptera[8], but since in these cases heterochromatin is not differentially eliminated on the basis of parental origin these are not examples of uniparental genetic systems. The mechanism of X dosage compensation in marsupials and some extra-embryonic tissues of placental mammals however, exhibits many of the features of a uniparental genetic system determined by sex of the parent. In contrast to the usual mechanism of X dosage compensation of placental mammals in which one or other of the two X chromosomes is inactivated more or less randomly in each somatic cell, in marsupials the paternal X chromosome is preferentially inactivated (Cattenach, 1975; Cooper *et al.*, 1977; VandeBerg, 1983; VandeBerg *et al.*, 1983) This system therefore exhibits many of the characteristics of PGL.

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[5] Ciliates have two nuclei, a micronucleus which is passed between generations and a macronucleus which is made anew in each cell by a complex process of genomic rearrangement. During this process thousands of internal sequences, of mostly repetitive DNA are eliminated and the chromosomes are rejoined. These chromosomes then break up into smaller fragments at specific breakpoints and telomeres are than added so that the overall DNA content of the macronucleus is not only smaller than that of the micronucleus but is also distributed amongst a larger number of chromosomes (Steinbrück, 1986; MacGregor, 1993). Similar chromosome breakage and telomere addition also occurs in the nematode worm *Ascaris* (Müller *et al.*, 1991) in which internal DNA elimination deletes a gene coding for a putative ribosomal protein (Etter *et al.*, 1991).

[6] The phenomenon (now known as chromatin diminution) was first identified by Theodore Boveri in the nematode worm *Parascaris equorum* (then known as *Ascaris megalocephala*) (Boveri, 1887). See also Tobler (1986), Tobler *et al.* (1992), Pimpinelli and Goday (1989), Gilbert (1991), Goday *et al.* (1992), MacGregor (1993) and Müller (1995). For an alternative interpretation see Nieuwkoop and Sutasurya (1981).

[7] See Beerman (1959; 1977).

[8] Heitz (1934) noticed that centromeric heterochromatin was underrepresented in polytene cells of *Drosophila*. This, however, has tended to be interpreted in terms of differences in the degree of replication rather than elimination of chromatin (see Spradling and Orrweaver (1987) for a review). Karpen and Spradling (1990) and Spradling *et al.* (1993) have demonstrated that this phenomenon is in fact due to active elimination rather than underreplication and suggest that due to certain molecular similarities between this and other superficially dissimilar examples of chromatin elimination, this may be an evolutionarily conserved developmental mechanism which has not been widely detected and that many other examples may still await discovery.

> *"Since in most cases, cellular genes do not appear to be lost, the phenomenon has been relegated to the status of an evolutionary curiosity, perhaps a nettlesome manifestation of "parasitic" DNAs that are able to excise themselves so as to prevent any deleterious effects on somatic cell activities. Although many possible functions have been suggested for DNA elimination (reviewed by Tobler, 1986), there has been a tendency to focus on potential functions for the eliminated material in the germ line. This overlooks the possible importance of the elimination process itself, rather than the material removed. Elimination changes the structure of somatic genomes and may do so in a manner that varies between different cell types. The modifications might modulate gene function by controlling the general organisation of DNA within the nucleus. This seems particularly likely with regard to genes such as ribosomal genes, that are frequently located in heterochromatic regions containing repetitive sequences."*
>
> *Allan C. Spradling, Gary Karpen, RobertGlaser, Ping Zhang , 1993*

Some lower dipterans (Cecidomyidae and Sciaridae) have extremely complicated genetic systems in which entire chromosomes are expelled from somatic and germ line cell lineages in various ways some of which conform to the model of a uniparental genetic system determined by the sex of the parent (see White (1973), Gerbi (1986) and MacGregor (1993)). For more information on the genetics of Sciaridae see Metz (1925; 1926; 1927; 1931; 1938), Metz and Schmuck (1929; 1932), Metz and Smith (1931), Du Bois (1932), Reynolds (1938), Berry (1941), Crouse (1943; 1960; 1979), Amabis and Janczur (1978), Stocker (1978), Amabis *et al.* (1979), Cestari and Simoes (1980), Eastman *et al.* (1980), Abbott and Gerbi (1981), Abbott *et al.* (1981), Lima-da-Silva (1981), Rubin (1981), Wei *et al.* (1981), Abbott (1982), Busen *et al.* (1982), Casartelli and Basile (1982), Glover *et al.* (1982), Kubai and Hong (1982), Zaha *et al.* (1982), Amabis (1983), Iwata (1983), Perondini *et al.* (1983), Pessacq-Asenjo (1984), Zaha *et al.* (1984), Dessen and Perondini (1985; 1991), Gerbi (1986), Perondini *et al.* (1986), Ruder *et al.* (1987), Perondini and Dessen (1988), DiBartolomeis and Gerbi (1989), Kerrebrock *et al.* (1989), Recco-Pimentel *et al.* (1989), Lara *et al.* (1991), Perondini and Otto (1991), Ribeiro and Perondini (1991), Santelli *et al.* (1991) and Paco-Larson *et al.* (1992). For information on uniparental genetic systems in the Cecidomyidae see White (1946, 1950), Suomalainen (1950), Swanson (1957) and Bacci (1965).

The *Drosophila* mutant *pal*^{1} for the gene *paternal loss inducer* (*pal*) satisfies all of these criteria except for the last (Baker, 1975). The effect of this mutant appears to be mediated by non-disjunction rather than by heterochromatisation, however, since all of the other criteria are satisfied this mutant represents the best single-gene model of PGL identified to date. (Other *Drosophila* mutants including those for the genes *claret*, *Horka*, *mitotic loss inducer*, *no distributive disjunction*, and *nonclaret disjunctional* all cause genome loss (mediated by non-disjunction) but satisfy fewer of the criteria for a single-gene model of PGL than *paternal loss inducer.*

PGL can be induced by intracellular *Wolbachia* bacteria in the autoparasitoid wasp *Nasonia vitripennis* see (Hunter, Nur and Werren, 1993; Reed and Werren, 1995; Werren *et al.*, 1995). The fact that this occurs may indicate that PGL had some role in the normal ancestral genetic system of this group, a prediction of the hypothesis of Schrader and Hughes-Schrader (1931) that PGL is an intermediate step in the evolution of arrhenotoky from ancestral diplodiploidy.

An important distinction between prefertilisation and postfertilisation uniparental genetic systems is that in prefertilisation systems (with the exception of those with pseudogamy) mating is not necessary in order to produce offspring whereas in postfertilisation systems it is essential. Any hypothesis therefore, which attempts to explain the evolution of uniparental genetic systems on the basis of the advantage inherent in the lack of requirement for a mate may account for prefertilisation systems but will not explain postfertilisation systems within these classes.

The Timing of Genome Loss

Postfertilisation uniparental genetic systems can be classified according to the timing of chromatin elimination. In systems with germ line genome loss the genome of the biparental parent is retained in an active state in somatic tissues (which are therefore diploid) but is eliminated from the germ line just prior to, or during, gametogenesis whereas in systems with somatic genome loss the genome of the biparental parent is lost early in embryogenesis with the consequence that adult uniparental offspring are somatically haploid.

Uniparental genetic systems with somatic genome loss include all systems in which the genome of the biparental parent is lost early in the embryogenesis of uniparental offspring with the consequence that adults are somatically haploid. For example in somatic pseudoarrhenotoky males loose their paternal genome early in embryogenesis (but not usually until after a few rounds of mitosis (Nelson-Rees, Hoy and Roush, 1980)) with the consequence that adult males are somatically haploid. This occurs in many species of mites as well as those members of the superfamily Coccoidea (Homoptera) with the Diaspidid genetic system.

Uniparental genetic systems with germ line genome loss include all systems in which the uniparental offspring retain the genome of the biparental parent in an active state in somatic tissues (which are therefore diploid) but these are eliminated from the germ line just prior to, or during, gametogenesis. For example in germ line pseudoarrhenotoky males retain the paternal genome in an active state in somatic tissues (which are therefore diploid) but these are eliminated from the germ line just prior to, or during, spermatogenesis. This occurs in those members of the superfamily Coccoidea with the Comstockiella and Lecanoid systems. The species determined uniparental genetic system of *Rana esculenta* is a germ line postfertilisation system *Rana esculenta* since the elimination of the genome of the biparental parent occurs in spermatogonial and oogonial cells.

Pseudogamy

Prefertilisation uniparental genetic systems can be divided into two classes on the basis on whether or not the gamete produced by the uniparental parent requires activation by a gamete from the biparental parent. Systems which require such an activation demand that mating takes place despite the lack of syngamy, e.g., when sperm are required to activate eggs but the paternal genome is not incorporated into the zygote (Kiester, Nagylaki and Shaffer, 1981; Kirkendall and Stenseth, 1990; Stenseth, Kirkendall and Moran, 1985). This phenomenon is called **pseudogamy**[9] (meaning 'false marriage'), a term introduced

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[9] A pseudogamic maternal daughters systems is found in *Rhabditis monohystera* (Nematoda: Rhabditida) (Belar, 1923; Belar, 1924) but see (Nigon, 1949a; Nigon, 1949b)) which produces amphimictic eggs, from which both males and females arise, and pseudogamous eggs, which only give rise to females (Nigon, 1947). This genetic system is likely to have arisen from the same XX/XO that gave rise to arrhenotoky in the Oxyurida and also gave rise to the genetic system of the genus *Caenorhabditis* which contains selfing sequential hermaphrodites in which sperm are produced first and then stored after which the gonad becomes an ovary for the rest of the adult life producing eggs which it fertilises with its own stored sperm. XXAA, XXXAA, XXXAAAA and XXXXAAAA genotypes are hermaphrodite but XA, XXAAA and XXAAAA remain male for their entire lives (Madl and Herman, 1979; Nigon, 1949a; Nigon, 1949b; Nigon, 1951a; Nigon, 1951b)). In nematode worms of the genus *Rhabditis* pseudogamy is associated with both hermaphroditism (monoecy) and gonochorism (dioecy): *R. aberrans* (Kruger, 1913) and *R. anomala* (Hertwig, 1922) are hermaphrodites whilst *R. pellio* (Hertwig, 1920), *R. leptodora*, *R. longicauda* (Hertwig, 1922) and *R. monohystera* (= bellari) (Belar, 1923; Belar, 1924) are gonochoric. Hybrids of the nematode worms *Heterodera trifolii* and *H. schachtii* (females of *H. trifolii* and males of *H. schachtii*) also exhibit pseudogamy as do the tetraploid (hermaphrodite) form of oligochaete worms of the subspecies *Allolobophora caliginosa trapezoide* (Omodeo, 1952; Omodeo, 1955) and the oligochaete worms *Lumbriculatus lineatus* (Christensen, 1960; Christensen and OConnor, 1958) and *Cognettia glandulosa* (Christensen, 1961). In *L. lineatus* there are two kinds of (hermaphroditic) individuals: diploid (2n=26) and triploid (3n=39); triploid individuals are pseudogamous, their eggs only developing if activated by the sperm of the diploid form which *"only stick to the surface and do not enter the egg"* (pseudofertilisation) (Christensen, 1980). Females of the springtail *Onychiurus procampatus* (Hexapoda: Collembola) are dimorphic. Small females lay small eggs which always give rise to small males and small females. Large females lay large eggs which give rise to large females only. Hale (1964) has shown that large females will only lay eggs when males of *O. procampatus* or *O. tricampatus* are present, however, in the presence of *O. tricampatus* males, no *tricampatus* characters were found in the offspring suggesting that true fertilisation had not taken place and that there had been no fusion of the nuclei or exchange of genetic material. Other arthropods in which pseudogamy has been demonstrated include the triploid leafhopper *Muellerianella fairmairei* (Booji, 1981; Booji and Guldemond, 1984; Drospopoulos, 1976) and hybrids of the spider beetles *Ptinus latro* (= *mobilis*) and *P. claviceps* (= *hirtellus*)(Moore, Woodroffe and Sanderson, 1956; Sanderson, 1960; Sanderson and Jacob, 1957). *P. claviceps* is diploid (2n=18) and gonochoric with both males and females whereas *P. latro* is triploid (3n=37) and consists only of females which can only reproduce if paired (pseudogamously) with males of *P. claviceps*. Sex-ratio (SR) strains of the Canadian bark beetles *Ips tridens*, *I. englemani*, *I. simirosteris*, *I. amiskwiensis* and *I. yahoensis* give rise to all female progeny. It has been suggested that these five species should be considered a single species with polymorphic females since males are indistinguishable cytologically and morphologically (Lanier and Oliver, 1966). Males with sisters of one normal (non-SR) morphological form, when mated with females of another give bisexual progeny which are morphologically variable in a manner suggesting that true syngamy has taken place. The (all female) offspring of males mated with SR females, however,

in 1881 by the botanist Focke (1881) and is a better description of this phenomenon than alternative term **gynogenesis** (meaning 'female descent') since any form of female parthenogenesis necessarily involves a female only lineage. The term pseudogamy could equally well apply to situations in which sperm activate eggs which then lose their own genome which is replaced by that of the sperm (**pseudogamic androgenesis**) which would distinguish this process from **androtoky** (the direct development of an individual from a male gamete without prior interaction with a female gamete. Although rare, pseudogamy occurs in a wide variety of organisms including many species of plants (Gustafsson, 1946; Nygren, 1954), is frequently associated with interspecific hybrids and polyploids and can occur in conjunction with any type of parthenogenesis.

Although pseudogamy has traditionally been associated with parthenogenesis it may also be associated with androgenesis if sperm require union with an egg before they can develop androgenetically. This may occur when eggs become overripe (or induced by irradiation of the egg) leading to degeneration of the maternal genome (Corley-Smith, Lim and Brandhorst, 1996; Parsons and Thorgaard, 1985; Purdom, 1969; Yamazaki, 1983). In this case the nuclear genome is contributed exclusively by the father but the extranuclear genomes come from the mother as normal.

It may, at first, seem that a pseudogamous female has the worst of both worlds since she gets neither the advantages associated with sex (generation of genetically variable offspring etc.) nor the advantage of being asexual that she can reproduce without having to find a mate. However, pseudogamy may reduce the costs of finding a mate, by allowing her to mate with males of other species, to an extent sufficient to overcome the loss of the advantages associated with sex, particularly if these advantages are small in the ecological milieu of the species concerned. An examination of the comparative ecology of pseudogamous organisms may shed some light on this matter.

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always resemble their mothers rather than the female relatives of their fathers. This suggests that the male has not contributed any genetic material to the offspring. Pseudogamous strains have also been found in the species *I. borealis* and *I. perturbatus*. The moth *Luffia lapidella* (Narbel-Hoffstetter, 1963; Narbel-Hoffstetter, 1964) is bisexual and diploid but has pseudogamous strains which have all female progeny. The fall cankerworm (*Alsophila pometria*) (Lepidoptera: Geometridae) (Mitter and Futuyama, 1977), a pest of apple and other fruit trees over much of North America, is also pseudogamous. Pseudogamy has been suggested in the Scorpion genus *Tityus* (White, 1973) to account for the presence of complex meiotic configurations made up of odd numbers of chromosomes but this has yet to be confirmed. Amongst the vertebrates pseudogamy has been demonstrated in the silver carp (*Carassius auratus gibelio*) (Berg, 1961; Cherfas, 1966; Lieder, 1959), the genbuna (*Carassius auratus langsdorfii*) (Kobayashi, 1971) and hybrids of fish in the genera *Poeciliopsis* and *Poecilia* (= *Mollienisia*) (*Poeciliopsis lucida/LLM* and *Poeciliopsis monacha/LMM* (Schultz, 1967; Schultz, 1969; Schultz, 1973; Schultz, 1977) and *Poecilia formosa/latipinna* (Hubbs, 1955; Hubbs, 1964; Hubbs and Hubbs, 1932; Kallman, 1962). Schultz has found two triploid hybrids of *Poeciliopsis lucida* and *Poeciliopsis monacha*. *LLM* consists of two chromosome sets of *lucida* and one of *monacha* and requires sperm from *lucida* for egg activation although no DNA from these sperm is transferred to the egg. For this reason Schultz has called these pseudogamous hybrids "reproductive parasites". *LMM* consists of two chromosome sets of *monacha* and one of *lucida* and is a reproductive parasite of *monacha*. Hybrids of the North American salamanders *Ambystoma jeffersonianum* and *A. platineum* and those of *A. laterale* and *A. tremblayi* (MacGregor and Uzzell, 1964; Uzzell, 1963; Uzzell, 1964; Uzzell and Goldblatt, 1967; Wilbur, 1971) form a complex very similar to that of the genus *Poecilia*. *A. tremblayi* is triploid with two sets of *laterale* chromosomes and one of *jeffersonianum* (LLJ). *A. platineum* has one set of *laterale* and two of *jeffersonianum* (LJJ). *A. laterale* and *A. jeffersonianum* are diploid (2n=28) and bisexual. *A. tremblayi* usually requires sperm from *A. laterale* for activation of the egg although these sperm to not contribute genetic material to the offspring. Similarly *A. platineum* has a requirement for the sperm of *A. jeffersonianum*. At some localities, however, the triploid biotypes occur in the absence of their diploid associates indicating that pseudogamy can, in some cases, be dispensed with. See also Purdom (1969).

Apparent pseudogamy may even provide an opportunity for transfer of subgenomic quantities of DNA which may be available for recombination. The 'pseudogamous' hybrid fish, the Amazon Molly (*Poecilia formosa*) consists only of diploid females (Meyer, 1938) which are dependent on sperm of males of the related species *P. latipinna* or *P. mexicana* as a physiological trigger of embryogenesis (Hubbs, 1955; Hubbs, 1964; Hubbs and Hubbs, 1932; Kallman, 1962; Vrijenhoek, 1989). Haskins *et al.* (1960) and Rasch *et al.* (1965) have shown that fertilisation does occasionally take place and produces offspring with some paternal characteristics. These hybrid progeny are triploid, with one paternal and two maternal sets of chromosomes (Rasch, Prehn and Rasch, 1970). Schartl *et al.* (1995) have detected the incorporation of "microchromosomes" (subgenomic quantities of DNA) from *P. mexicana* into the genome of *P. formosa* and they suggest that this may compensate for the effect of Muller's Ratchet, the theory that since without recombination a genome can never produce offspring with fewer mutations than itself, deleterious mutations will accumulate in asexual clones leading eventually to their extinction (Felsenstein, 1974; Muller, 1964)[10]. Theoretical studies (Charlesworth *et al.*, 1993a; Charlesworth *et al.*, 1993b; Kondrashov, 1994) show that very small amounts of recombination may be sufficient to prevent extinction due to Muller's Ratchet and extend the life of an apparently asexual clonal lineage beyond that predicted by models based on the accumulation of deleterious mutations (10^4-10^5 generations (Gabriel *et al.*, 1993; Lynch and Gabriel, 1990)). There is some argument over whether microchromosomes can recombine with the maternal genome, and if so, the extent to which they could compensate for Muller's Ratchet (Beukeboom *et al.*, 1995). If recombination does take place then pseudogamy is not an appropriate term. The term pseudogamy should be reserved for those cases in which there is absolutely no opportunity for paternally derived genetic material to affect the phenotype of the offspring.

Pseudogamous production of males has frequently been invoked to account for obligatory mating in species with haploid males. However, this is also a feature of somatic pseudoarrhenotoky and in most cases in which pseudogamous arrhenotoky has been proposed, somatic pseudoarrhenotoky has not been ruled out. The differences are slight since mating is required in both cases and although somatic pseudoarrhenotoky permits transfer of paternally derived genetic material to male offspring there is usually no opportunity for recombination with the maternal genome until spermatogenesis by which time the paternal genome has been lost.[11]

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[10] However see Beukeboom *et al.* (1995) for an alternative interpretation.

[11] Pseudogamic production of males has been proposed to account for obligate mating in a number of groups of haplodiploid parasitiform mites, however in none of these cases has pseudogamy ever been proven and in two of these groups (the family Phytoseiidae (Helle *et al.*, 1978; Hoy, 1979) and the genus *Dicrocheles* (Otopheidomenidae) (Treat, 1965)) the genetic system has subsequently been shown to be somatic pseudoarrhenotoky, i.e., genuine syngamy has been shown to occur. This is also likely be true of those closely related mites with haplodiploidy and obligate mating in which the genetic system is yet to be discovered (i.e. Ascinae (Ascidae) except the arrhenotokous genus *Gamasellodes* (Walter and Ikonen, 1989; Walter and Lindquist, 1989), *Antennoseius janus* (Antennoseiidae) (Lindquist and Walter,

THE SEX DETERMINED UNIPARENTAL GENETIC SYSTEMS

The remainder of this review will be concerned exclusively with sexually determined uniparental genetic systems. To avoid unnecessary confusion, in this section, in which the general classes of uniparental genetic systems determined by sex are described, only the prefertilisation systems are listed in the main text. Each of these has a postfertilisation counterpart and these are listed in tables 1 and 2. Following this general discussion is a more lengthy treatment of each of the prefertilisation and postfertilisation systems. There are four basic uniparental genetic systems determined by parental sex. There are six ways of combing these four systems in groups of two but only four of these combinations describe meaningful composite genetic systems.

The Basic Sex Determined Uniparental Genetic Systems

Bull (1983) provided a classification of the four uniparental genetic systems in which one of the sexes is produced biparentally whilst the other is produced uniparentally. These are the four **basic systems** (table 1.1). He did not include systems in which both sexes are produced uniparentally. The systems Bull included in his scheme were:

- **Maternal sons** - any uniparental genetic system in which the uniparental parent is female and the uniparental offspring are male. These are by far the most common of the uniparental genetic systems and include arrhenotoky and pseudoarrhenotoky.

- **Paternal daughters** - any uniparental genetic system in which the uniparental parent is male and the uniparental offspring are female. This is a theoretical possibility although it has never actually been observed. The possibility that this is due to observational bias is discussed later.

- **Paternal sons** - any uniparental genetic system in which both the uniparental parent and the uniparental offspring are male. These systems have never been found in nature but in many groups with haploid males paternal son systems have not yet been ruled out.

- **Maternal daughters** - any uniparental genetic system in which both the uniparental parent and the uniparental offspring are female. Maternal daughter systems without males or in which males are infertile are common and referred to by the collective term thelytoky, however, maternal daughter systems in which fertile males are produced biparentally are rare. Such a system occurs in the nematode worm *Rhabditis monohystera.*

1989), *Podocinus sagax* (Podocinidae) (Wong, 1967), *Dermanyssus gallinae* (Dermanyssidae) (Oliver, 1965) but see (Warren, 1940) and *Haemogamasus ambulans* (Haemogamasidae) (Furman, 1959)).

Table 1.1: A Scheme for the Four Basic Uniparental Genetic Systems Determined by Parental Sex

Uniparental Parent	Uniparental Offspring	Genetic System	Prefertilisation System	Postfertilisation System
Female	Male	Maternal Sons	Arrhenotoky	Male PGL*
Female	Female	Maternal Daughters	(see table 1.3)	Female PGL
Male	Male	Paternal Sons	(see table 1.3)	Male MGL
Male	Female	Paternal Daughters	(see table 1.3)	Female MGL

* Pseudoarrhenotoky

Table 1.2: A Scheme for the Four Composite Uniparental Genetic Systems Determined by Parental Sex

Component Systems	Composite Systems	
	Prefertilisation	**Postfertilisation**
Unisexual Systems		
Maternal sons + Maternal daughters	Deuterotoky	Pseudodeuterotoky
Paternal sons + Paternal daughters	Androdeuterotoky	Pseudandrodeuterotoky
Bisexual systems		
Paternal sons + Maternal daughters	Homotoky	Pseudohomotoky
Maternal sons + Paternal daughters	Heterotoky	Pseudoheterotoky
Maternal sons + Paternal sons	Will give rise to a simple paternal sons system without females after a single generation	
Maternal daughters + Paternal daughters	Will give rise to a simple maternal daughters system without males after a single generation	

The Composite Sex Determined Uniparental Genetic Systems

To Bull's classification I have added four **composite systems** which are made up of pairs of basic systems. There are two **unisexual composite systems** in which individuals of both sexes are produced uniparentally by the same sex and two **bisexual composite systems** in which individuals of both sexes are produced uniparentally by parents of different sexes (table 1.2). In none of these four systems are any of the offspring produced biparentally.

There are two other logical possibilities for combining the basic uniparental genetic systems; these are maternal sons + paternal sons and maternal daughters + paternal daughters, however, these systems could never be biological realities. Maternal sons + paternal sons is not possible since no females would be produced and within a single generation the population would come to consist only of androgenetic males i.e. the maternal daughters component would be lost after the first generation giving rise to a simple paternal sons system with no females which would be indistinguishable from androtoky. Similarly maternal daughters + paternal daughters is not possible since no males would be produced and within a single generation the population would come to consist only of parthenogenetic females i.e. the paternal sons component would be lost after the first generation giving rise to a simple maternal daughters system with no males which would be indistinguishable from thelytoky.

Unisexual Composite Systems

In both of the uniparental composite systems only individuals of one sex reproduce. This means that offspring of the opposite sex to the parent are redundant unless they can disperse to a population in which they can reproduce, the population in which they arise has genetic system polymorphism or they increase the inclusive fitness of offspring of the reproducing sex in some way. In the absence of these opportunities for the non-

reproducing offspring to increase the fitness of a parent, unisexual composite system are likely to lose the non reproducing sex and revert to basic systems with only one sex (i.e. deuterotoky → thelytoky in which females give rise to females without the involvement of males and androdeuterotoky → androtoky in which males give rise to males without the involvement of females). The two unisexual composite systems are:

- **Deuterotoky** (= maternal sons + maternal daughters) - systems in which males and females are both produced uniparentally by females. Deuterotoky occurs infrequently in nature.

- **Androdeuterotoky** (= paternal sons + paternal daughters) - systems in which males and females are both produced uniparentally by males. Androdeuterotoky is unknown in nature.

Bisexual Composite Systems

Bisexual composite systems can also be referred to as **mixed systems** since they are the only uniparental systems which contain both parthenogenetic and androgenetic elements. The two bisexual composite systems are:

- **Homotoky** (= paternal sons + maternal daughters) - systems in which male offspring arise uniparentally from male parents whilst female offspring arise uniparentally from

female parents. Mitochondrial DNA inheritance in the blue mussel (*Mytilus*) is a homotokous system in which the paternal sons component is a germ line postfertilisation system the maternal daughters component is a prefertilisation system (Zouros *et al.*, 1994b) (see below).

- **Heterotoky** (= maternal sons + paternal daughters) - systems in which male offspring arise uniparentally from female parents whilst female offspring arise uniparentally from male parents. Heterotoky is unknown in nature.

Evolution of Composite Systems

Whilst the unisexual composite systems may have arisen in a single step it seems likely that for bisexual composite systems to evolve one of the two component genetic systems, the **primary genetic system**, must evolve first and the other, the **secondary genetic system**, must subsequently arise within a population which already has the primary genetic system. If the probabilities of a uniparental genetic system evolving are multiplicative then the evolution of composite systems is extremely unlikely, however, if there is a feature of the life history of an organism which predisposes it to uniparentalism in general then composite systems may be more likely to evolve since any population with a primary uniparental genetic system is likely to have this predisposing factor which will increase the probability that a second uniparental genetic system will evolve. Such a model presupposes that the selective advantages for all uniparental genetic systems are similar. For example in mitochondrial inheritance in *Mytilus* the maternal daughters component is probably primary and the paternal sons component secondary.

THE PREFERTILISATION SYSTEMS

The diversity of prefertilisation uniparental systems determined by parental sex (i.e. those in which the offspring of at least one sex arise have only one parent) is illustrated in table 1.3.

Parthenogenesis

Parthenogenesis can be defined as any system in which females produce offspring asexually i.e. eggs develop directly into a new individual without fertilisation. Traditionally three different types of parthenogenesis have been distinguished on the basis of the sex of the offspring produced parthenogenetically but other parthenogenetic also systems exist.

The Three Traditional Forms of Parthenogenesis

- **Arrhenotoky** in which males are produced parthenogenetically but females are produced zygogenetically

Table 1.3: A Scheme for the Prefertilisation Systems

Origin of Females	Origin of Males	Genetic System
Uniparental offspring of female	Uniparental offspring of female	Deuterotoky*
Uniparental offspring of female	Biparental	Anti-arrhenotoky
Uniparental offspring of female	Absent or infertile	Thelytoky
Biparental	Uniparental offspring of female	Arrhenotoky
Uniparental offspring of male	Uniparental offspring of male	Androdeuterotoky
Uniparental offspring of male	Biparental	Paternal daughters
Biparental	Uniparental offspring of male	Paternal sons
Absent or infertile	Uniparental offspring of male	Androtoky
Uniparental offspring of female	Uniparental offspring of male	Homotoky
Uniparental offspring of male	Uniparental offspring of female	Heterotoky
Biparental	Biparental	Biparentalism
Uniparental offspring of male	Absent or infertile	Not possible
Biparental	Absent or infertile	Not possible
Absent or infertile	Uniparental offspring of female	Not possible
Absent or infertile	Biparental	Not possible
Absent or infertile	Absent or infertile	Meaningless

*If males and females are parthenogenetically produced by *different* females then this is referred to as arrhenothelytoky.

- **Thelytoky** (= Female Parthenogenesis *sensu stricto*) in which females are produced parthenogenetically but fertile males do not occur
- **Deuterotoky** in which both males and females are produced parthenogenetically.

Two Other Forms of Parthenogenesis

- **Anti-arrhenotoky** - prefertilisation maternal daughter systems with biparental fertile males in which females are produced parthenogenetically but males are produced zygogenetically
- **Arrhenothelytoky** in which males and females are produced parthenogenetically by different individual females

Reclassification of Parthenogenesis

Using the traditional criterion for the classification of parthenogenetic systems (the sex of the offspring produced parthenogenetically) we can divide these five systems into three groups which are considered in turn in the next three sections. These groups are:

- **Male parthenogenesis** in which only males are produced parthenogenetically (arrhenotoky)
- **Female parthenogenesis (*sensu lato*)**[12] in which only females are produced parthenogenetically (thelytoky, anti-arrhenotoky)
- **Mixed parthenogenesis** in which both males and females are produced parthenogenetically (deuterotoky, arrhenothelytoky)

Each of these parthenogenetic systems may ~~be~~ either persist for a large number of generations, occur only occasionally in an otherwise zygogenetic population (**tycoparthenogenesis**) or alternate with zygogenetic generations (**cyclical parthenogenesis**).

Male Parthenogenesis

Male parthenogenesis consists of any genetic system in which males (and only males) are produced parthenogenetically e.g. arrhenotoky (including diploid arrhenotoky). If females exist then they arise zygogenetically.

Arrhenotoky

The term Arrhenotoky refers to any genetic system in which males arise from unfertilised eggs (prefertilisation maternal sons) whilst females arise from fertilised eggs. This is one of the three traditional types of parthenogenesis. Arrhenotoky has arisen only infrequently but has given rise to several large radiations (i.e. in the groups in which it occurs it is often characteristic of the entire group or at least a large part of it). This means that, in most cases, arrhenotoky is of ancient origin. This is in contrast to the widely scattered phylogenetic distribution and recent origin of thelytoky in most modern groups. This suggests that although arrhenotoky may be subject to greater constraints than thelytoky it is more evolutionarily stable.

Previously the term **haplodiploidy** has been considered to be a synonym of arrhenotoky (e.g. Bull (1983)), however Norton *et al.* (1993) have extended the definition of haplodiploidy to include somatic pseudoarrhenotoky which shares with arrhenotoky the property that males are haploid and females are diploid and that males only transmit the genome received from their mothers. Here I extended this definition still further to include any genetic system in which one sex is somatically diploid whilst the other is somatically haploid, this includes all prefertilisation systems (except for those such as diploid arrhenotoky in which the initially haploid genome is duplicated) and all forms of

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[12] The term female parthenogenesis has been treated by some authors as a synonym of thelytoky. Here I broaden the usage of the term to include all genetic systems in which females are produced parthenogentically. This includes not only thelytoky (= female parthenogenesis *sensu stricto*) but also anti-arrhenotoky.

somatic genome loss including somatic pseudoarrhenotoky but not germ line genome loss in which adults are somatically diploid. The term **male haploidy** is used to describe all uniparental genetic systems in which males are haploid (i.e. arrhenotoky, somatic pseudoarrhenotoky and somatic male MGL)[13]. Arrhenotoky is found in nematode worms[14], thrips[15], Hymenoptera[16], beetles[17], scale insects[18] and both acariform and parasitiform mites (see chapter 3).

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[13] Taxa in which males are haploid but the precise genetic system is unknown are found in the monogonant rotifers (Birky and Gilbert, 1971; Jones and Gilbert, 1977; Rishi and Kamaljeet, 1989a; Rishi and Kamaljeet, 1989b; Robotti, 1975; Walsh and Zhang, 1992), the Acari (Norton *et al.*, 1993) and the monofamilial Homopteran superfamily Aleyrodoidea (whiteflies) (Thomsen, 1927; Whiting, 1945). Male haploidy is a feature of both maternal son and paternal son systems but since paternal son systems are unknown in nature the genetic systems of all groups with haploid males have always been assumed to be maternal son systems, however, in very few of these cases have paternal son systems been ruled out. Male haploidy has been identified in a number of species of rotifer associated with complex life cycles alternating sexual and asexual generations (figure 1.3) and characterised by dwarf and structurally degenerate males restricted to particular seasons or environmental conditions (Lucks, 1929). These studies have focused on cytophotometric measurements of relative DNA content (Jones and Gilbert, 1977) as well as simple chromosome counts (Rishi and Kamaljeet, 1989a; Rishi and Kamaljeet, 1989b; Robotti, 1975; Walsh and Zhang, 1992) however sex specific markers have not been used and therefore it is not known which of the three haploid male systems (arrhenotoky, somatic pseudoarrhenotoky or somatic male MGL) is responsible. Male haploidy is by no means the genetic system of all of the rotifers, or indeed all of the sexual rotifers (the rotifers include a number of asexual species including one group (the bdelloids) which are thought to have been asexual for over 35 million years (Judson and Normark, 1996; Poinar and Ricci, 1992; Ricci, 1987)). A robust phylogeny as well as sex specific marker studies of genetic systems within this group are needed to identify the haploid male clades before more can be said about the origin of male haploidy in this group. In 1973 M. J. D. White, in his influential book *"Animal Cytology and Evolution"* (White, 1973) was forced to conclude that *"....the literature on the cytology of rotifera is in a rather chaotic state, there being so many points of disagreement between the various accounts that it is difficult to arrive at any firm conclusions"*. Whilst a great deal of progress has been made since then the field is still characterised by confusion rather than consensus (but see Birky and Gilbert (1971)).

[14] Arrhenotoky occurs in the pinworms (Nematoda: Oxyurida) (Adamson, 1989) and is likely to be the genetic system of this entire clade of parasitic worms. The outgroup and parsimony criteria predict that the plesiotypic state of a character is likely to be that found in the nearest outgroup (Kitching, 1992a). The plesiotypic genetic system in the Oxyurida is therefore likely to be the genetic system of its nearest outgroup which is probably the free living Rhabditida. These have an XX/XO system of sex determination (Nigon, 1965; Walton, 1959) so it is likely that arrhenotoky arose only once in the common ancestor of all the Oxyurida from an XX/XO system of sex determination in association with a shift from a free living to a parasitic lifestyle. The pinworms (Nematoda: Oxyurida) consist of about 130 genera in two superfamilies; the Thelastomatoidea, which are all endoparasites of arthropods, and the Oxyuroidea, which are all endoparasites of vertebrates. The monophyly of the order is well established and characterised by a number of synapomorphies (Adamson, 1989). Male haploidy has been established in 18 species in 9 genera representing both superfamilies. In the Oxyuroidea male haploidy has been identified in all three families; in the Pharyngonidae, in nine species parasitic on tortoises (*Tachygonetria conica*, *T. dentata*, *T. macrolaimus*, *T. numidica*, *T. longicollis*, *T. setosa*, *T. pusilla*, *Mehdiella microstoma*, and *M. uncinata* (Adamson and Petter, 1983a)), two species parasitic on lizards (*Tachygonetria vivipara* and *Thelandros alatus* (Adamson and Petter, 1983b)) and a single species of frog parasite (*Gyrinicola batrachiensis* (Adamson, 1981c), in the Oxyuridae, in one species parasitic on lagomorphs (*Passalurus ambigua* (Adamson, 1989)) and one species parasitic on mice (*Syphacia obvelata* (Adamson, 1984b)) and in the Heteroxynematidae, in a single species also parasitic on mice (*Aspiculuris tetraptera* (Adamson, 1984b)). Of the five families of Thelastomatoidea, male haploidy has been identified in only one, the Thelastomatidae, in two species parasitic on diplopods (*Hammerschmidtiella andersoni* and *Thelastoma* sp.(Adamson, 1984a)) and one species parasitic on cockroaches (*Hammerschmidtiella diesingi* (Adamson and Nasher, 1987)). Adamson (1989) also considers illustrations of *Auchenacantha robertrauschi* and *A. parva* in Hugot (1986), which show eggs in the uterus with either one or two pronuclei, to be evidence of haplodiploidy in these species although there is no evidence in this case that it is the male eggs which have one pronucleus and the female eggs which have two. Evidence that male haploidy in this order is due to arrhenotoky comes from the observation of male progeny production by virgin females in the (unkaryotyped) species *Protrellus dixoni*, a parasite of cockroaches in New Zealand (Zervos, 1988). Although there are a number of earlier reports of diploid males in pinworms (Goswami, 1976a; Goswami, 1976b; Goswami, 1977; Walton, 1924; Walton, 1959), Adamson (1989) gives good reasons for considering these to be in error.

[15] Arrhenotoky is probably the genetic system of all of the sexual thrips (Bull, 1983; Davidson and Bald, 1931; Risler and Kempter, 1962; Shull, 1914; Shull, 1917; White, 1973). The most primitive of the modern families of Thysanoptera is the Aeolothripidae (Ananthakrishnan, 1979) and it seems likely that this family evolved from a psocid like ancestor sometime in the Palaeozoic (Karny, 1922). The oldest fossil thysanopteran is *Karataothrips jurassicus* from the Upper Jurassic and Sharov (1972) suggests that this has close affinities with Permian fossil psocids of the extinct family Lophioneuridae (the smallest of all the psocids) and that the ancestor of the Thysanoptera therefore arose from within this family no earlier than the beginning of the Triassic. Arrhenotoky must therefore have evolved at some point along the lineage Lophioneuridae (Psocoptera) → Karataothripidae (Thysanoptera) → Aeolothripidae (Thysanoptera). Thrips

Diploid Arrhenotoky

Diploid arrhenotoky occurs when males arise from unfertilised eggs as in true haplodiploid arrhenotoky and begin to develop as haploids but then become diploid through fusion of the first two haploid cleavage nuclei (Nur, 1972). The only known example of this system occurs in soft scales of the family Coccidae (Homoptera: Coccoidea) in which one of the two sets of chromosomes becomes heterochromatised after a few rounds of cell division (Phillips, 1965). Since the two genomes are identical such systems do not gain the advantages of diploidy associated with the presence in a cell of two different genomes (e.g. heterozygote advantage), but they may gain some other advantage such as the opportunity for mismatch repair.

Female Parthenogenesis

Female parthenogenesis consists of any genetic system in which males (and only females) are produced parthenogenetically e.g. thelytoky and anti-arrhenotoky. If males exist then they arise zygogenetically.

Thelytoky

Thelytoky (= female parthenogenesis *sensu stricto*) is characterised by the development of females and occasional 'spanandric' males (which are always infertile) from unfertilised eggs. If fertile males are produced then this should not be referred to as thelytoky but as deuterotoky (if the males are produced parthenogenetically) or anti-arrhenotoky (if they are produced zygogenetically)[19]. Thelytoky has arisen many times

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have highly asymmetric mouthparts and Grinfel'd (1959) considers this to be an adaptation acquired by the first thrips for feeding on pollen grains. (This would have been gymnosperm pollen prior to the evolution of angiosperms in the mid Cretaceous.) It therefore seems that thrips evolved from a group of psocids that took to eating pollen during the Palaeozoic and it was presumably at this point that they acquired arrhenotoky. The thrips have since radiated into a variety of phytophagous niches and some species have even become predators of mites, psocids, coccids, white flies, aphids, leaf hoppers, tingids and other thrips but all modern groups have retained arrhenotoky.

[16] With the exception of a few secondary thelytokes, deuterotokes and arrhenothelytokes, arrhenotoky is probably the genetic system of the entire Hymenoptera (Crozier, 1975).

[17] There appear to have been three independent origins of arrhenotoky in the Coleoptera; one in the family Micromalthidae which contains only one species, *Micromalthus debilis* and belongs to the primitive suborder Archaeostomata (Scott, 1936; Scott, 1941), and two in the subfamily Scolytinae of the family Scolytidae (bark beetles) which is a highly derived family of the suborder Polyphaga; one in the tribe Xyleborini and another in the tribe Dryocetini (Kirkendall, 1993). *Micromalthus debilis* has four kinds of reproductive females; adult females, arrhenotokous paedogenetic larvae producing only male progeny parthenogenetically, thelytokous paedogenetic larvae producing only female offspring parthenogenetically and deuterotokous paedogenetic larvae producing both male and female offspring parthenogenetically. Males of this species are haploid whilst females are diploid.

[18] Arrhenotoky occurs in five closely related genera of giant scales (Margodidae) (Hughes-Schrader, 1948).

[19] In the triploid parthenogenetic race of the isopod crustacean *Trichoniscus elisabethae* ('forme' *coelebs*), triploid males are produced sporadically by parthenogenesis however, although these males copulate with females no fertilisation takes place and the eggs develop parthenogenetically. Other thelytokous groups producing small numbers of infertile males parthenogenetically include the diploid and tetraploid parthenogenetic races of *Artemia salina*, many parthenogenetic races of phasmids (White, 1973) and tycothelytokous tettigids (Orthoptera) (about 0.2% in *Apotettix eurycephalus* (Suomalainen, 1950)). Some parthenogenetic groups however produce very few males. Amongst the thelytokous weevils (Coleoptera: Curculionidae), for example, only in *Strophosomus melanogrammus* have exceptional males ever been identified. The *Daphnia pulex* complex (= *D. pulex sensu stricto, D. pulicaria, D. middendorffiana* and

during the evolution of most groups of plants and animals (notable exceptions include the gymnosperms and the mammals). With the exception of a few ancient asexual clades (Judson and Normark, 1996)[20], thelytoky in extant groups has had a recent origin (Seiler, 1943; Seiler, 1946) therefore, although thelytoky has arisen frequently and must be subject to less severe constraints than arrhenotoky, it is much less evolutionarily stable.

Anti-Arrhenotoky

I introduce the term anti-arrhenotoky to refer to all prefertilisation uniparental genetic systems in which females are produced asexually and males are produced sexually since this is identical to arrhenotoky but with the sexes of the offspring reversed. These systems are distinct from true thelytoky in which fertile males never occur and from deuterotoky in which males are produced parthenogenetically rather than zygogenetically.

Anti-arrhenotoky occurs in the nematode worm *Rhabditis monohystera,* in association with highly female biased sex ratios, in which females appear to produce two different types of ova. In one of these the second meiotic division is suppressed and it must be activated pseudogamously by sperm which degenerate after entering the egg leading to parthenogenetic production of a uniparental female. The other, in which the second meiotic division is normal, must be fertilised and gives rise to a biparental male following fusion of the egg and sperm pronuclei (Belar, 1923; Belar, 1924). Nigon (1949a; 1949b) reported a variation on this system in the same species in which half of the biparental offspring were female.

Mixed Parthenogenesis

Mixed parthenogenesis consists of any genetic system in which both males and females are produced parthenogenetically e.g. deuterotoky and arrhenothelytoky.

Deuterotoky

Deuterotoky (= amphitoky) consists of the parthenogenetic production of both fertile males and females (Beatty, 1957; Suomalainen, 1950). This differs from anti-arrhenotoky since in deuterotoky males are produced parthenogenetically whereas in anti-arrhenotoky they are produced zygogenetically. The term deuterotoky should be reserved for those

D. tenebrosa) sporadically produce males apomictically which are genetic replicates of their mothers (Haney and Buchanan, 1987). Weider and Hobaek (1997) have reported higher clonal richness in Siberian populations of the *Daphnia pulex* complex containing males in conjunction with deviations from the Hardy-Weinberg genotype frequencies expected in thelytokous populations which are characteristic of sexually reproducing populations, suggesting that sexual reproduction is occurring within these populations. Dufresne (1995) has reported occasional sexual reproduction in this complex in the N. W. Territory of Canada and Weider and Hobaek (1997) suggest that this, together with the observation of frequent production of males in N. W. Alaska (see Weider and Hobaek (1997) for references) indicates that the area around the Bering Sea known as Beringia is a centre of sexual reproduction in this circumarctic species complex serving as a hotspot for increased clonal diversity fuelling the production of novel clones.

[20] Such as the bdelloid rotifers which are thought to have been asexual for over 35 million years (Judson and Normark, 1996; Poinar and Ricci, 1992; Ricci, 1987) and some groups of oribatid mites which may have been asexual for over 200 million years (Norton and Palmer, 1991).

cases in which fertile males (which can give rise to their own offspring) are produced parthenogenetically. In cases where sterile males are produced, even in large numbers, the term thelytoky should be used.

Facultative deuterotoky occurs in the mayfly *Centroptilium luteolum* (Ephemeroptera) in which unfertilised eggs give rise to progeny of both sexes (Degrange, 1956). Deuterotoky occurs in paedogenetic larvae of the primitive beetle *Micromalthus debilis* (Coleoptera: Micromalthidae), and is often found in association with cyclical parthenogenesis, e.g. in some of the more primitive gall wasps (Hymenoptera: Cynipidae). Mittler (1946) was able to induce deuterotoky in virgin females of the white-fly *Trialeuroides vaporarium* which are normally arrhenotokous by subjecting them to high temperatures (about 38°C). This altered the maturation process leading to production of females due to the retention of the diploid number of chromosomes.

Arrhenothelytoky

I introduce the term arrhenothelytoky to refer to the parthenogenetic production of males and females by different females, i.e., the population contains both maternal daughter and maternal son systems but these are not found together in the same individual. This differs from deuterotoky since in arrhenothelytoky no single female produces both male and female offspring[21]. Arrhenothelytoky occurs in some of the more advanced gall wasps (Hymenoptera: Cynipidae) in association with cyclical parthenogenesis and seems to have evolved from deuterotoky in this family[22].

Cyclical Parthenogenesis

A life cycle characterised by alternating generations of parthenogenesis and zygogenesis is referred to as cyclical parthenogenesis. A sexual generation gives rise to a parthenogenetic generation by amphimixis which may give rise to subsequent parthenogenetic generations by thelytoky but which always eventually gives rise to another sexual generation by deuterotoky, arrhenothelytoky or arrhenotoky. It is a typical feature of cyclical parthenogenesis that in groups in which it occurs it is characteristic of

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[21] Females which produce only male offspring are referred to as **arrhenogenous**, those which produce only female offspring as **thelygenous** and those which produce both as **amphogenous** whether these offspring are produced parthenogenetically or zygogenetically.

[22] Arrhenothelytoky and deuterotoky are found in the family Cynipidae (the gall wasps) in association with cyclical parthenogenesis. Deuterotoky is found some of the more primitive members of this family e.g. *Andricus operator austrior* (Patterson, 1928) but this has been replaced by arrhenothelytoky in some of the more advanced gall wasps e.g. *Neuroterus baccarum* (Dodds, 1939), *N. lenticularis* (Doncaster, 1911) and *N. irregularis albipleurae* and *Belenocnema tretae kinseyi* (Patterson, 1928). These arrhenothelytokes have spring generations which consist of parthenogenetic females of two types; arrhenotokes which produce only males and thelytokes which produce only females. Since male and female offspring are produced by different individuals this is a case of arrhenothelytoky rather than true deuterotoky. In arrhenotokous females a normal meiosis takes place leading to the production of haploid males whereas in thelytokous females there is no meiosis and diploid females are produced. It seems that true deuterotoky is ancestral to arrhenothelytoky i.e. sex determination appears to have shifted backwards one generation (Doncaster, 1910; Doncaster, 1916).

the entire group suggesting that it is evolutionarily very stable (Bell, 1982). Rapid reproduction during the thelytokous phase is often accelerated by **paedogenesis** (asexual propagation by sexually immature individuals) (e.g. in Trematoda, Cecidomyidae and *Micromalthus*).

Cyclical parthenogenesis can occur in conjunction with male haploidy (e.g. in Rotifera , Cecidomyidae, *Micromalthus*, and Cynipidae) or male diploidy (e.g. Trematoda, Cladocera and Aphididae) (Lucks, 1929; Suomalainen, 1950). Various environmental factors have been implicated in the switch from parthenogenesis to zygogenesis (Shull, 1929). In the Cladocera temperature (both high and low), availability of food and population density have been shown to be important factors (Banta *et al.*, 1939; Mortimer, 1936) whilst in the rotifera there is some difference of opinion on this matter (Buchner, 1937; Luntz, 1926; Weber, 1930). Some species of aphids (i.e. the trimorph species such as *Phylloxera caryaeculis*) have only three generations per year, the cycle being fixed and not influenced by environmental factors, while in others life cycle switching is controlled by temperature and periodicity of illumination (Lawson, 1939). Lees (1960) has demonstrated the operation of an "interval timer" in the aphid *Megoura viciae*. This requires that the asexual cycle proceeds for a certain period of time (rather than number of generations) before the conditions which would otherwise cause the switch to sexuality can operate.

Androgenesis

Androgenesis can be defined as the development of a new individual from a male gamete without fertilisation. This occurs naturally in hybrid stick insects of the genus *Bacillus* (Mantovani and Scali, 1992; Scali *et al.*, 1995; Tinti and Scali, 1996) and has been artificially induced in *Drosophila* (Komma and Endow, 1995) and many species of fish and amphibians (Corley-Smith *et al.*, 1996; Parsons and Thorgaard, 1985; Purdom, 1969; Yamazaki, 1983).

Androgenesis can be classified in a similar way to parthenogenesis i.e. mixed androgenesis (androdeuterotoky in which both males and females arise androgenetically), female androgenesis (paternal daughter systems in which females are produced androgenetically and males are biparental) and male androgenesis (paternal son systems in which males are produced androgenetically and females biparentally (the androgenetic equivalent of anti-arrhenotoky) and androtoky in which males are produced androgenetically and females are either absent or infertile (the androgenetic equivalent of thelytoky) (table 1.3).

There is a much greater constraint on the evolution of androgenesis than on the evolution of parthenogenesis. This means that it is much more difficult for males to become prefertilisation uniparental parents than for females. Uniparental genetic systems in which

the uniparental parent is male are therefore much less evolutionarily stable than those in which the female is the uniparental parent because females can achieve evolutionary stability by evolving towards prefertilisation uniparentalism. If there is no substantial difference between the barriers to androgenesis and parthenogenesis then many of the conclusions of this review will have to be substantially revised, however the relative frequencies of parthenogenesis and androgenesis in nature strongly suggest that this is not the case.

Mixed Systems

In the prefertilisation forms of each of the two mixed systems, two separate lineages would arise which would be reproductively isolated from each other and would not exchange any genetic information. In homotoky one lineage would consist of only females arising by thelytoky whilst the other would consist of only males arising by androtoky. In heterotoky both lineages would consist of alternating males and females arising by parthenogenesis and androgenesis respectively. Since these two lineages would be reproductively isolated they would constitute separate species. This lineage separation would also be true of prefertilisation mixed systems in which the genomes inherited by the uniparental offspring do not recombine prior to elimination of the genome of the biparental parent. Since recombination usually occurs only during gametogenesis and elimination of the genome of the biparental parent usually occurs prior to gametogenesis, this is unlikely to be the case. For example, mitochondrial DNA inheritance in *Mytilus* is a mixed system with a prefertilisation maternal daughters component and a germ line postfertilisation paternal sons component in which the two genomes are found together in male germ line cells prior to spermatogenesis but since the two types do not recombine they remain genetically isolated. This is reflected in a substantial degree of sequence divergence between the two types.

THE POSTFERTILISATION SYSTEMS

Each of the different types of parthenogenesis has a postfertilisation counterpart. The postfertilisation counterpart of arrhenotoky, for instance, is called **pseudoarrhenotoky**. This refers to any postfertilisation uniparental genetic system in which the paternal genome of initially diploid male embryos is eliminated at some stage prior to spermatogenesis. Previous authors have used the terms **parahaploidy** and paternal genome loss (PGL) as synonyms for pseudoarrhenotoky. Here I have broadened the definitions of these terms to apply them to all uniparental genetic systems. PGL refers here to any postfertilisation uniparental genetic system in which the paternal genome is lost, i.e. all postfertilisation maternal systems. The definition of the term parahaploidy has been extended to include any uniparental genetic system in which the uniparental offspring inherits the genome of the biparental parent i.e. this term is now synonymous with postfertilisation uniparentalism.

Pseudoarrhenotoky occurs infrequently and has not given rise to large radiations such as those associated with arrhenotoky. Arrhenotoky and pseudoarrhenotoky are, however, often found together in taxa, suggesting that pseudoarrhenotoky may be an intermediate step in the evolution of arrhenotoky from diplodiploidy (Schrader and Hughes-Schrader, 1931). Evidence for this comes from the work of (DeJong, Lobbes and Bolland, 1981) who have identified a heterochromatic chromosome arm, which may be the vestiges of the heterochromatised complement of a pseudoarrhenotokous ancestor, in the arrhenotokous mites *Geolaelaps aculifer* and *Stratiolaelaps miles* (Mesostigmata: Laelapidae) which belong to a group (the Dermanyssina) known to contain diplodiploid, pseudoarrhenotokous and arrhenotokous members. There may therefore be some taxic variation in the degree of heterochromatisation with a gradual loss of the heterochromatised portion of the genome as arrhenotoky evolves from pseudoarrhenotoky. There may also be variation in the timing of the loss of the paternal genome with somatic pseudoarrhenotoky evolving from germ line pseudoarrhenotoky as the heterochromatised paternal genome is lost earlier and earlier in ontogeny. This is highly speculative, however, and to date there is no evidence to support it. Pseudoarrhenotoky occurs in beetles[23], scale insects[24] and parasitiform mites[25].

In some pseudoarrhenotokes paternal genome loss may be incomplete. Perrot-Minot and Navajas (1995) have presented evidence that in the pseudoarrhenotokous mite *Typhlodromus pyri* some of the paternal genome may be retained. This could be due to partial retention of the paternal set of inactivated chromosomes in some tissues in haploid males but another possibility which they consider is the potential existence of a paternally derived B chromosome which escapes paternal genome loss in male embryos and segregates with the maternal chromosomes. Such a B chromosome has been reported in the lecanoid system of the mealybug *Pseudococcus affinis* (Nur and Brett, 1988) and similarly in the pseudogamous fish, the *Poecilia formosa* (Schartl *et al.*, 1995).

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[23] Pseudoarrhenotoky has been reported in the Scolytid beetle; *Hypothenemus hampei* (the coffee berry borer)(Borsa and Kjellberg, 1996; Brun *et al.*, 1995).

[24] The lecanoid system (L system) of the mealy bugs (Pseudococcidae) (Hughes-Schrader, 1948; Schrader, 1923) is a pseudoarrhenotokous system with germ line genome loss in which the chromosomes of paternal origin are heterochromatised in male embryos (Brown and Nelson-Rees, 1961). These heterochromatic chromosomes are destroyed following prophase I of spermatogenesis. Another system, the Comstockiella system (C system), found in the felted scales (Eriococcidae) (Schrader, 1929), is identical to the L system except that the heterochromatised chromosomes are destroyed just prior to prophase I of spermatogenesis (Brown, 1963). Another pseudoarrhenotokous system is found in the armoured scales (Diaspididae) and has therefore been called the diaspidid system (D system). In the D system the paternal genome is eliminated in early cleavage at about the same time that heterochromatisation occurs in the L and C systems (Brown and Bennett, 1957). In this respect the D system resembles the somatic pseudoarrhenotokous genetic system of the mite family Phytoseiidae. It seems likely that the D system has evolved several times from the C system which in turn evolved from the L system which along with haplodiploid arrhenotoky arose from an ancestral XX/XO sex chromosome system with heterogametic males. Diploid arrhenotoky may have arisen from either of the L or C systems (Nur, 1980).

[25] Although pseudoarrhenotoky has been reported in a number of acariform mites, none of these reports have ever been confirmed. For details see Chapter 3.

MITOCHONDRIAL DNA INHERITANCE

Mitochondrial DNA (mtDNA) is usually inherited maternally (Avise and Lansman, 1983; Avise and Vrijenhoek, 1987; Gyllensten, Wharton and Wilson, 1985; Hayashi *et al.*, 1978; Lansman *et al.*, 1983; Wilson *et al.*, 1985) and is therefore a good example of a uniparental inheritance system (although a low level of leakage of paternal mtDNA may be more common than previously thought). Notable deviations from this norm do exist for example observation of heteroplasmy for divergent mtDNA molecules has led to the identification of paternal transmission of mtDNA in *Drosophila* (Kondo *et al.*, 1990; Kondo *et al.*, 1992), hybrid mice (Gyllensten *et al.*, 1991), mussels of the genus *Mytilus* (Zouros *et al.*, 1992), the sturgeon (Brown *et al.*, 1992) and the anchovy (*Engraulis encrasicolus*) (Magoulas and Zouros, 1993). Biparental inheritance has been shown to follow cell fusion experiments in *Drosophila simulans* (Satta *et al.*, 1988) and selective propagation has led to paternal inheritance of mitochondria in a redwood tree (*Sequoia semperivirens*) (Neale *et al.*, 1989). Paternal mtDNA is transmitted to female honeybees but this is lost during development and is not passed on to subsequent generations (Meusel and Moritz, 1993) (mitochondrial PGL).

Early evidence for maternal transmission of mitochondrial DNA came from *Xenopus* (Dawid and Blackler, 1972) and donkey (Hutchison *et al.*, 1974) however neither of these studies ruled out low levels of paternal contribution. Complete penetration of the spermatozoan into the egg often results in incorporation of mitochondria; this is particularly common in insects. In taxa with polyspermy (penetration of the egg by more than one sperm) the amount of paternal mtDNA transferred to the egg may be considerable (Birky, 1978). Polyspermy occurs in many taxa including reptiles (Rothschild, 1956), birds (Hamilton, 1952) and insects (Davey, 1965). This phenomenon has been particularly well documented in the honeybee in which more than ten sperm may enter a single egg (Petrunkewitsch, 1901). These accessory sperm have even been found to replicate resulting in gynandromorph individuals with both male and female tissue (Drescher, 1963; Rothenbuhler, 1957). Paternal mtDNA degenerates quickly in the egg, representing 25% of the total mtDNA of the egg 12 hours after fertilisation but only about 2.5% of the total mtDNA of the larvae 96 hours after fertilisation. Recombination of mtDNA has not been reported in insects and so there is little opportunity for paternal mtDNA to make a significant long-term contribution, however, theoretical studies (Chapman *et al.*, 1982; Takahata and Maruyama, 1981; Takahata and Palumbi, 1985) show that even very low levels of paternal leakage could have a substantial effect. This may be important since studies of africanised honeybees, for example, have tended to assume a strict maternal inheritance of mitochondria (e.g. Sheppard, 1991). Even in isogamous fungi (*Physarium polycephalum*) in which mtDNA does recombine (Kawano *et al.*, 1991) mtDNA still seems to be inherited uniparentally (Meland *et al.*, 1991).

Of particular interest is the mitochondrial inheritance system in *Mytilus* in which females inherit mtDNA uniparentally from their mothers, passing it on to both daughters and sons, whilst males inherit mtDNA biparentally passing only the mtDNA which they received from their fathers to their sons (Skibinski, Gallagher and Beynon, 1994; Zouros *et al.*, 1994a; Zouros *et al.*, 1994b). This is an example of homotoky in which the paternal sons component is a postfertilisation system (germ line maternal mitochondrial genome loss) whilst the maternal daughters component is a prefertilisation system. Since the paternal sons component is a *germ line* postfertilisation system, somatic cells will contain two types of mitochondria (heteroplasmy), however, since the genomes of these two different types of mitochondria do not recombine the maternal and paternal mitochondrial lineages never exchange genetic material. This has led to 10-20% sequence divergence between these two mitochondrial lineages (Fisher and Skibinski, 1990; Hoeh *et al.*, 1991). Hurst and Hoekstra (1994) cite this as evidence in favour of the hypothesis that uniparental inheritance of mitochondria has arisen as an adaptation to prevent the spread of selfish genetic elements.

Chapter Two

Uniparental Genetic Systems and the Evolution of Haplodiploidy. II. Evolutionary Advantages

"The peculiarities of haplodiploidy suggest that considering the selective factors which cause its evolution is critical in developing a comprehensive view of how sexual systems operate and in understanding the elusive adaptive basis of sexuality."

Gerald Borgia, 1980

SUMMARY

Can a critical examination of the hypotheses available to account for the evolution of haplodiploidy can be aided by considering haplodiploidy as one particularly successful example of a whole class of uniparental genetic systems? A number of potential advantages for the evolution of haplodiploid genetic systems are reviewed in the wider context of uniparental genetic systems as a whole to help explain the apparent lack of uniparental genetic systems other than those with a 'maternal sons' or 'maternal daughters' patterns of inheritance. Maternal son and maternal daughter systems are common in nature whereas paternal son and paternal daughter systems are rare. This is because of greater constraints on androgenesis than on parthenogenesis. Postfertilisation uniparental genetic systems are able to stabilise conflict between paternal and maternal genomes because once the genome of the biparental parent is no longer inherited by the uniparental offspring there is no opportunity for direct conflict. However, since constraints on androgenesis are so high the switch from prefertilisation to postfertilisation uniparentalism is asymmetric. Prefertilisation systems with paternal sons or paternal daughters would be much less likely to achieve this stabilising switch than those with maternal sons or maternal daughters which would mean that postfertilisation systems with maternal sons and maternal daughters would be much more common than those with paternal sons and paternal daughters, a prediction which fits well with our current knowledge of uniparental genetic systems in nature. This model would also predict that prefertilisation systems would be less biased towards maternal systems, a prediction which is less well supported by available evidence although likely to be subject to a large degree of observational bias. Prefertilisation systems are more likely to be discovered in taxa which also have postfertilisation systems since more effort is made to understand the genetic systems of groups which are already interesting. Since these prefertilisation systems are phylogenetically related to the postfertilisation systems which alerted researchers to the potential interest of the group and since these postfertilisation systems

are likely to be maternal systems, considerable observational bias is to be expected unless two conditions are satisfied:

- Phylogenetic relationships are explicitly taken into account when making hypotheses about the evolution of uniparental genetic systems. (Since all organisms are related, samples of taxa can never be truly independent of each other but a phylogenetic approach to comparative biology will correct for this non-independence (Felsenstein, 1985b; Harvey and Pagel, 1991)).

- Unbiased sampling of taxa.

INTRODUCTION

The study of the evolution of haplodiploidy suffers from many of the same problems as the study of the evolution of sex. In both cases there are a great many hypotheses but very few empirical data to support any of these more strongly than any other. Kondrashov (1993) listed 20 different hypotheses for the evolution of sex. Here I describe a 13 different hypotheses which have been proposed to explain the evolution of male haploidy. Although all of these hypotheses were originally proposed in the context of maternal son systems it is worth considering their potential applicability to other uniparental genetic systems. Since uniparental genetic systems other than female parthenogenesis and maternal son systems are rare, any selective force likely to favour the evolution of maternal son systems without similarly favouring other uniparental systems is more likely to be important than one which would favour any uniparental genetic system. If forces equally likely to favour any uniparental genetic system over ancestral diplodiploidy were important then (accounting for observational bias) all uniparental genetic systems should be equally frequent in nature. Since there are many hypotheses purporting to explain the evolution of maternal son systems this approach may be a valuable way of assessing the relative importance of each.

POTENTIAL CONSTRAINTS ON THE EVOLUTION OF UNIPARENTAL GENETIC SYSTEMS

Evolutionary advantage alone is not sufficient to ensure the evolution of a potential innovation if there are genetic or developmental factors preventing it from arising in the first place. Such factors are known as constraints. Here I briefly examine 9 potential constraints on the evolution of uniparental genetic systems before going on to discuss in greater depth each of the 13 hypotheses proposed to explain the evolutionary advantages of those systems able to overcome these constraints.

Constraints on the evolution of all uniparental genetic systems

Gametogenesis

A specialised mode of gametogenesis must arise in the uniparental offspring so that only the genome from the uniparental parent is passed on.[26] Since gametogenesis is modified in all uniparental systems this is a potential constraint on all such systems. Wrensch *et al.* (1994) have suggested that inverted meiosis is a prerequisite for the evolution of haplodiploidy. Inverted meiosis is itself constrained by a requirement for holokinetic chromosomes (John, 1990).

Constraints on the evolution of prefertilisation uniparental genetic systems

Initiation of Development in Unfertilised Gametes

This will not be an obstacle to the evolution of postfertilisation uniparental genetic systems in which genuine fertilisation takes place but it is a potential barrier to the evolution of prefertilisation uniparental genetic systems. Initiation of development in unfertilised eggs is unlikely to be restrictive in groups in which thelytoky is common since thelytokes are also subject to this constraint and the high frequency of thelytokes in many taxa indicates that they have easily overcome this particular impediment on a great number of occasions (Peacock, 1944; Tyler, 1941) i.e. the scattered phylogenetic distribution of thelytoky suggests that this potential constraint is not an important one.[27] Initiation of development of unfertilised sperm, however, is likely to be a very strong constraint on the evolution of androgenesis since sperm do not normally contain all of the cellular components necessary to develop into an entire adult multicellular organism. This means that whilst uniparental genetic systems with parthenogenetic components may be common those with androgenetic components (i.e. prefertilisation systems with paternal sons or paternal daughters) will be rare. In pseudogamic systems egg development is stimulated by sperm without transfer of any genetic material. This may be due to a constraint on egg development which requires some physiological or biochemical trigger from sperm in order for the egg to develop successfully.

Sex Determination

A specialised mode of sex determination must arise in order for a prefertilisation uniparental genetic system to evolve. Such a system may be more easily derived from

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[26] For a discussion of spermatogenesis in the Hymenoptera see Suomalainen (1950) and Crozier (1975).

[27] Thelytoky is not, however, common in all groups with haplodiploidy. Thelytoky is rare in nematodes. Early reports of parthenogenesis in some soil dwelling nematodes (Nigon, 1965) actually represent cases of hermaphroditism (Adamson, 1989). There are a few genuine cases; *Strongyloides* spp. are cyclical parthenogens and a number of species of *Tylenchida* parasitic on cultivated plants are genuine thelytokes but elsewhere in the Nematoda the ecological requirements for the ability to found a new colony from a single individual and to begin reproduction at the onset of sexual maturity without having to find a mate has been fulfilled by hermaphroditism rather than thelytoky (e.g. *Caenorhabditis elegans*) (Adamson, 1989). This suggests that the inability to initiate development in the absence of fertilisation may have been a constraint on the evolution of haplodiploidy in this group which the Oxyurida had to overcome.

some systems than others. White (1973), for example, suggested that haplodiploidy has arisen from an Xn0 sex determination system with few autosomes and that these autosomes have been incorporated into the sex chromosomes. There are two different types of genetic (as opposed to environmental) sex determination; these are genic balance systems and dominant Y/Z systems (Charlesworth, 1991; White, 1973). All XX/XO systems must be genic balance systems whereas XX/XY and ZW/WW may be either genic balance[28] or dominant Y/Z systems[29]. Maternal son systems seem to have always arisen in group with genic balance systems e.g. XX/XO in the Oxyurida, Coccoidea, and Mesostigmata and (probably) a genic balance XX/XY in Xyleborini (White, 1973).[30] Since postfertilisation uniparental genetic systems provide an opportunity for normal sex determination prior to elimination of the genome of the biparental parent, this will not be a constraint on such systems. In postfertilisation systems the sex may be determined by the offspring or by either parent[31] and sex determination may be secondary to elimination of the biparental genome or vice versa. In prefertilisation systems the sex must be determined by the uniparental parent and is a consequence of whether or not fertilisation takes place.[32]

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[28] Genic balance systems are found in *Caenorhabditis elegans* (Akerib and Meyer, 1994; Ellis and Kimble, 1994; Meyer, 1988; Villeneuve and Meyer, 1990), *Lymantria dispar* (the gypsy moth) (which has a ZW/WW system of sex determination = female heterogamety) (see Goldschmidt (1923) and what Goldschmidt (1920; 1942; 1955), Schrader and Hughes-Schrader (1931) and Bull (1983) have to say about the theory that the egg contains both male and female determining elements but that in haploid males the male determining genes exert their effect prior to maturation and thus confer male determining properties to the egg cytoplasm), *Drosophila* (Albrecht and Salz, 1993; Baker and Belote, 1983; Barbash and Cline, 1995; Bridges, 1921; Bridges, 1939; Chase and Baker, 1995; Cline, 1985; Cline, 1993; Horabin and Schedl, 1995; Nöthiger and Steinmann-Zwicky, 1987; Paroush *et al.*, 1994; Patterson and Stone, 1952; Steinmann-Zwicky, Amrein and Nothiger, 1990) and various insects with XX/XO systems (White, 1973).

[29] Dominant Y/Z systems constitute those systems in which the presence of a chromosome determining the heterogametic sex is dominant over all other sex chromosomes i.e. in a dominant Y system any individual with at least one Y chromosome (or sex determining region of the Y chromosome) will be male, all other individuals being female by default whilst in a dominant Z system all individuals with at least one Z chromosome (or sex determining region of the Z chromosome) will be female, all other individuals being male by default. Dominant Y systems are found in *Homo sapiens* and other mammals (White, 1973) (Capel, 1995; Graves and Foster, 1994; Hunter, 1995; Marx, 1995a; McElreavey *et al.*, 1995; Schafer, 1995), the tipulid fly *Pales ferruginea* (Ullerich, Bauer and Deitz, 1964), and the calliphorid flies *Phormia regina* and *Lucilia cuprina* (Ullerich, 1963). A dominant Z system is found in *Bombyx mori* (the silk moth) (Tazima, 1964).

[30] It would seem sensible to suggest that haploid male systems are more likely to arise in groups in which haploids are male than those in which haploids are female. If groups in which haploids are male are more prone to uniparentalism then this may explain the higher incidence of uniparental systems with haploid males than those with haploid females found in nature.

[31] Although if sex were determined by the biparental parent this would lead to sex ratios highly skewed towards the sex of the biparental offspring since the loss of representation of genes of the biparental parent in uniparental offspring would offset the costs of investing too heavily in one sex. Since the uniparental parent would benefit from a 1:1 sex ratio (or a sex ratio biased in favour of the sex of the uniparental offspring due to increased genetic representation in the next generation), the two sexes will be in conflict over the optimal sex ratio.

[32] During the early part of this century a number of hypotheses were made concerning the chromosomal mechanism of sex determination and it was seen as important that these hypotheses could explain why males would be haploid in haplodiploid species. In 1909 Wilson asserted that it is the total quantity of X chromatin present in the cell of a developing zygote which determines its sex i.e. two X chromosomes contain the amount of chromatin which will cause the development of a female whereas a single X chromosome contains the amount of chromatin which will cause development of a male (Wilson, 1909). In such a system haploids would be male. By 1922, however, C. B. Bridges had realised that autosomes were also important in sex determination and was able to point out that.... *"In chromosome constitution the intersexes [of* Drosophila*] differ from females only in that they have an extra set of autosomes. This proves that the autosomes are concerned with the determination of sex."* (Bridges, 1922). Witschi (1929)assumed that diploids have two copies of a female determining gene F and two of a male determining gene M. Since diploids are

Genomic Imprinting

Postfertilisation uniparental genetic systems require that genomes are treated differently according to their parental origin. The molecular mechanism by which uniparental parents achieve heterochromatisation of the genome of the biparental parent in the uniparental offspring in postfertilisation uniparental genetic systems is unknown but the parental origins of each chromosome must be imprinted upon it in order for selective heterochromatisation to occur. Genomic imprinting is therefore a prerequisite for the evolution of postfertilisation uniparental genetic systems.[33]

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always female he concluded that, due to epistasis, the genes for femaleness must dominate i.e. FF > MM. In haploids however, physiological differences between haploid and diploid calls change the conditions such that there is a reversal of epistasis and the single copy of the male determining gene dominates the single copy of the female determining gene i.e. M > F. This however does not explain sex determination but merely restates the problem in terms of cell physiology. The determinant of sex has now shifted from ploidy to some aspect of cell physiology which covaries with ploidy but Witschi has nothing to say about what this may be. Presumably this reversal of epistasis in different cellular environments is genetically determined in which case the gene responsible for this reversal is the true sex determining gene and epistatic reversal is merely the mechanism by which it performs its sex determining role. C. B. Bridges, in a series of papers written between 1925 and 1930, proposed that, in *Drosophila* at least, X chromosomes are female determining and autosomes male determining (Bridges, 1925a; Bridges, 1925b; Bridges, 1930). According to this hypothesis, sex is determined by the ratio of the number of X chromosomes (X) to the number of haploid sets of autosomes (A) i.e. sex is determined according to the value of X/A. A ratio of 1 or more is characteristic of femaleness and a ratio of 0.5 or less is characteristic of maleness. (Ratios between these values would correspond to intersexuals.) According to this hypothesis however, a haploid would have 1X and 1A and the ratio would therefore be 1, i.e. a haploid would be expected to be female. Although haploid *Drosophila* are inviable, using flies which were mosaic for the loss of varying numbers of chromosomes, Bridges was able to show that the regions which had lost the most chromosomes and were therefore the closest to being haploid (he was unable to demonstrate complete elimination of a haploid chromosome set) appeared to be female in character, for example, if the front leg was such a region then the sex comb, a structure found only in the male, would be absent (See Komma and Endow (1995) for more recent evidence that haploid *Drosophila* are female). This model, called the genic balance model, is an example of a class of models called ratio models in which sex is determined according to the ratio of two quantities (see Goldschmidt (1934) for another example). Schrader and Sturtevant (1923) agreed with Bridges that X chromosomes are female determining and autosomes are male determining and that sex is determined by a balance of these forces, however, they calculated this balance of forces differently and this had different consequences. They gave an arbitrary value of -6 to each X chromosome and +2 to each autosome set, the threshold value for femaleness was set at -7 and maleness at -5 and then sex was determined according to the algebraic sum of these values for all the chromosomes of a cell. (Values between -7 and -5 would correspond to intersexuals.) In this system a haploid (1X + 1A) would have a value of -4 and would therefore be male. This model is a particular example of a class of models called the additive value models which vary only in the values they ascribe to the chromosomes and thresholds. These values correspond to hypotheses of gene dosage effects. Depending on how these numbers are chosen, models can be constructed which explain any set of predictions for the sexual phenotypes of different genotypes. (Other additive value models can be found in Goldschmidt (1920), Heslop-Harrison (1919), Winge (1932; 1934), Yamamoto (1969) and Bulmer and Bull (1982).) Bridges criticised the algebraic sum hypothesis on the basis that the differences between the algebraic sums of different genotypes are not proportional to the differences in their phenotypes, for example, there is a difference of eight units between diploid and tetraploid females which look almost identical but a difference of only six units separates diploid males and diploid females. The genic balance hypothesis has a much greater degree of correspondence between genotype and phenotype (diploid and tetraploid females have the same value) but cannot account for haploid males. Ratio models can be transformed into additive value models by taking the logs of the ratios. The additive value model corresponding to the genic balance hypothesis of Bridges predicts that sex is determined according to the quantity log X - log A. A value for this parameter greater than or equal to zero would correspond to a female and a value less than -0.3 would correspond to a male with intersexuals representing the intermediate values. A haploid in this model (1X + 1A) would have a value of zero and hence would be female. For general reviews of sex determination see Haldane (1957), Bacci (1965), Crew (1965), McLaren (1981), Hodgkin (1990; 1992), Bownes (1992), Parkhurst and Meneely (1994), Hunter (1995), Marx (1995b), Ryner and Swain (1995) and Mittwoch (1996). The only haplodiploid group in which the mechanism of sex determination has been investigated is the Hymenoptera. For a historical perspective on sex determination in Hymenoptera see Crozier (1975). For further more general information on sex determination in Hymenoptera see Whiting (1943a; 1943b), Suomalainen (1950), Rothenbuhler (1957), Kerr (1974), Crozier (1971; 1977), Bull (1981), Cook (1993a; 1993b), Periquet *et al.* (1993) and Cook and Crozier (1995).

[33] A number of hypotheses have been suggested in order to account for the evolution of genomic imprinting. It may have evolved in order to prevent the parthenogenetic development of the unfertilised egg with no contribution from a male. This would be of advantage only to genes which cannot be passed on by females i.e. selfish genetic elements on the Y chromosome. There is also likely to be conflict between maternal and paternal genomes in terms of the transfer of nutrients from mother to offspring since it is the interests of the mother to restrict growth of the foetus to some extent so that other foetuses fathered by different males (either concurrently or subsequently) can develop fully whilst it is in the

Constraints on the evolution of haplodiploid genetic systems

Deleterious Alleles

The evolution of haplodiploid uniparental genetic systems is constrained by the expression of deleterious alleles in the hemizygous state in haploid individuals. This will not be a constraint on the evolution of germ line postfertilisation uniparental genetic systems in which all individuals are diploid. It will be a constraint on diploid arrhenotoky (since although males may be diploid they are nevertheless homozygous at all loci) but since diploid arrhenotoky is likely to be a modification of haplodiploid arrhenotoky this constraint will already have been overcome.

Population Structure

Haplodiploid uniparental genetic systems are unlikely to evolve in groups with high degrees of heterozygosity (Borgia, 1980; Brown, 1964).

Haploid Inviability

The evolution of haplodiploid uniparental genetic systems may be constrained by the inviability of haploid individuals (Schrader and Hughes-Schrader, 1931). This will not be a constraint on the evolution of germ line postfertilisation uniparental genetic systems in which all individuals are diploid.[34]

Dosage Compensation

A specialised mode of dosage compensation must arise in order for a haplodiploid genetic system to evolve (Trivers, 1988).

Constraints on the evolution of arrhenotoky

Hybridisation

Whiting (1945) suggested that arrhenotokes arise from hybridisation between diplodiploid and thelytokous forms. This would mean that arrhenotoky is more likely to

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interests of the father to promote the growth of the foetus of which he is the father at the expense of foetuses fathered by other males with which he shares no genetic interest (Moore and Haig, 1991). This predicts that imprinted genes are likely to be involved on growth regulation. Whilst this is true of some imprinted genes (e.g. *IgF2* and *IgF2R*) it is not true of others (e.g. *SmN* and *U2afbp-rs* which are involved in RNA splicing and have no obvious role in growth regulation). It has also been suggested that imprinting may have no function whatever and that it is in fact a relic of an ancient defence mechanism for inactivating foreign DNA (Barlow, 1993). More information on genomic imprinting can be found in Lyon and Rastan (1984); Monk and Surani (1990); Lyon (1993); Barlow (1994; 1995); Nicholls (1994); Latham *et al.* (1995); Latham (1996); Neumann and Barlow (1996); Martin (1996).

[34] There are two types of diploidy, **primary diploidy**, e.g. ancestral diplodiploidy, germ line postfertilisation uniparentalism and the diploid state of biparental offspring in uniparental genetic systems, and **secondary diploidy**, e.g. in males with diploid arrhenotoky in which a reversal to diploidy has occurred (females of the same species will be primary diploids). In systems with secondary diploidy this constraint must have been overcome in the past so although it may be important in the evolution of haploidy from primary diploidy but is unlikely to be so in the evolution of haploidy from secondary diploidy (i.e. a second reversal to haplodiploid arrhenotoky from diploid arrhenotoky).

evolve in groups in which thelytoky is common, however, this may be true anyway since groups in which thelytoky is common are groups in which the potential barrier to the evolution of arrhenotoky caused by the requirement for initiation of egg development in the absence of sperm is easily overcome.

EVOLUTIONARY ADVANTAGES OF UNIPARENTAL GENETIC SYSTEMS

Many of the hypotheses proposed to account for the evolution of haplodiploidy are able to explain both arrhenotoky and pseudoarrhenotoky although some of these (the developmental rate hypothesis, the deleterious allele hypothesis and one of the inbreeding hypotheses; the sexual variation hypothesis) will not explain germ line pseudoarrhenotoky i.e. they will only account for haplodiploid genetic systems. The facultative parthenogen hypotheses on the other hand, cannot account for the evolution of pseudoarrhenotoky at all since they rely on a property of arrhenotoky that is not true of pseudoarrhenotoky, namely that females can produce (male) offspring without mating. The gamete selection hypothesis will only explain the evolution of uniparental genetic systems in which males are the uniparental offspring. The repair hypothesis explains why pseudoarrhenotoky may be favoured over arrhenotoky in some cases. This information is summarised in table 2.1.

Hypotheses consistent with all uniparental genetic systems

Four hypotheses, the chromosomal drive hypothesis, the sex ratio hypothesis and one of the inbreeding hypotheses, the sexual variation hypothesis, suggest that there are equal selective advantages for any uniparental genetic system over ancestral diplodiploidy since the advantage accrues as a direct consequence of the transmission genetics of the system which is the same for all uniparental genetic systems. Since uniparental genetic systems are not all represented equally in nature, if any of these hypothetical advantages are important forces in genetic system evolution then the apparent unequal distribution of these systems must be due to some other factor such as constraints or observational bias.

The Chromosomal Drive Hypothesis

The uniparental inheritance of extranuclear genomes (anisogamy) may have arisen as a response to intragenomic conflict (Cosmides and Tooby, 1981; Grun, 1976; Hastings, 1992a; Hastings, 1992b; Hoekstra, 1987; Hurst, 1990; Hurst *et al.*, 1992). This may also be true of genetic systems in which nuclear genomes are inherited uniparentally. Maternal genes will increase their representation in haploid sons of maternal origin in comparison to diploid sons of biparental origin. This will double the probability of gene identity by descent between a grandmother and grandchild (Bucci *et al.*, 1990; Bull, 1979; Bull, 1983). There is therefore a selective pressure on females to cause heterochromatisation of the paternal chromosomal complement of their offspring in order to capitalise on this advantage. This means that pseudoarrhenotokous mutants will increase in frequency in a

Table 2.1: A Taxonomy of the Potential Advantages of Uniparental Genetic Systems

Hypothesis	Genetic Systems
Chromosomal Drive Hypothesis	All uniparental genetic systems
Sex Ratio Hypothesis	All uniparental genetic systems
Inbreeding Hypotheses	
Variable Gamete Hypotheses	
Variable Offspring Hypothesis	All uniparental genetic systems
Gamete Selection Hypothesis	All uniparental son systems
Inbreeding Depression Hypothesis	All haplodiploid genetic systems
Heterozygote Advantage Hypothesis	All haplodiploid genetic systems
Developmental Rate Hypothesis	All haplodiploid genetic systems
Hemizygosity Hypotheses	
Deleterious Allele Hypothesis*	All haplodiploid genetic systems
Beneficial Allele Hypothesis*	All haplodiploid genetic systems
Facultative Asexuality Hypotheses	
Population Density Hypothesis	All prefertilisation systems
Oedipal Mating Hypotheses	
Reproductive Success Hypothesis	Arrhenotoky only
Colonisation Hypothesis	Arrhenotoky only
Repair Hypothesis	All postfertilisation systems

*Both group and individual selectionist versions

diplodiploid population. There is, however, conflict between the maternal genes, which are selected to cause heterochromatisation of the paternal genes, and the paternal genes, which are under selective pressure to avoid this heterochromatisation, i.e. there is an evolutionary arms race between these two sets of chromosomes. This conflict will only affect genes tightly linked to sex determining loci since genes not so linked would spend an equal amount of time in both sexes.[35] If the maternal chromosomes win there will be evolution towards arrhenotoky under which the paternal genome will no longer be able to compete. If the paternal chromosomes win there will be reversion to diplodiploidy, i.e. pseudoarrhenotoky is an unstable intermediate between the much more stable states of diplodiploidy and arrhenotoky. This should be reflected in the phylogenetic relationships between these systems and would explain the lack of radiations associated with pseudoarrhenotoky. If reversion to diplodiploidy were more common than progression to

[35] Sex determining regions of the genome are often characterised by very low rates of recombination e.g. the sex determining portion of Y chromosome of humans. This would tighten the linkage between genes determining sex and those driving the evolution of uniparentalism and also facilitate the evolution of new types of sex determination associated with the switch in genetic system.

arrhenotoky then it could also explain the low frequency of evolution of maternal son systems in general (in comparison to diplodiploidy under a strong selective pressure towards pseudoarrhenotoky rather than in comparison to other uniparental genetic systems) although this could just as easily be explained by constraints on the evolution of these systems. This hypothesis cannot, however, account for long term (and therefore, presumably, evolutionarily stable) pseudoarrhenotoky in the mites and scale insects. In this model prefertilisation uniparental systems evolve by a two step process and it is conceivable that the bias towards maternal systems occurs at either step. Chromosomal drive may cause the evolution of all sorts of postfertilisation systems but it may be the switch to prefertilisation uniparentalism which is biased in favour of maternal systems. Furthermore, if postfertilisation systems either rapidly evolve into prefertilisation systems or revert to diplodiploidy then the stability of postfertilisation systems may also be biased such that paternal postfertilisation systems are more likely to revert to diplodiploidy than maternal ones increasing the relative length of time which maternal systems have to evolve into prefertilisation systems.

Why should the female always cause heterochromatisation of the paternal genome in her sons only? She could cause heterochromatisation of the paternal genome in her daughters rather than her sons leading to a maternal daughters system and this would still increase the representation of her genes in the next generation. This could ultimately lead to prefertilisation uniparentalism i.e. parthenogenetic production of daughters under which males would still be produced sexually but females would not require them for reproduction. It seems probable that such a system would ultimately lead to elimination of males and the species would become thelytokous. If there is no progression to parthenogenetic production of females the requirement for males of the same species with which to mate may still be relaxed under a postfertilisation maternal daughter system since the sperm of males of other species may be sufficient to stimulate egg development even if they are not sufficient for genuine syngamy. This may be the reason why pseudogamy is stable in some species. Elimination of conspecific males from such a population would result in pseudogamy with females becoming reproductive parasites of males of another species. Maternal daughter systems may therefore be rare because they are likely to evolve quickly into other systems (pseudogamy in postfertilisation systems and thelytoky in prefertilisation systems).

A female could cause heterochromatisation of the paternal genome in both sons and daughters. This is likely to lead to pseudogamy and ultimately (possibly via deuterotoky) to thelytoky since any advantage she may receive from the opportunity for her genome to recombine with that of the male and increase the genetic variability of her offspring would be lost and yet she would still not be able to capitalise on the advantage of not requiring a mate. Since there are organisms in which pseudogamy appears to be a stable state this is not always necessarily a problem but the general trend once the advantage of

recombination (whatever that may be) has been lost is likely to be towards thelytoky which would allow her to be free of the necessity to find a mate before she can reproduce. Such systems are therefore likely to be rare since they will quickly evolve into other systems (i.e. pseudogamy and ultimately thelytoky).

The male is similarly under selective pressure to cause heterochromatisation of the maternal genome in his offspring and thus increase the representation of his genes in the next generation. He may be able to induce maternal genome loss in his offspring, however, he has a problem that the female does not have. In order to take this inactivation of the maternal genome to the ultimate stage of omitting fertilisation altogether he must first overcome the considerable constraints on androgenesis. All paternal systems must be parahaploid unless the male can become androgenetic. Such systems are therefore inherently unstable and likely to be rare since they are will revert to diplodiploidy once the female acquires a mutation which will allow her to escape the effects of chromosomal drive.[36]

The Sex Ratio Hypothesis

In maternal son genetic systems a female can take control of the sex ratio of her offspring. In arrhenotoky this is achieved by selective fertilisation of each egg but in pseudoarrhenotoky it occurs by heterochromatisation of the paternally derived genome of the zygote. Sabelis and Nagelkerke (1988) have provided a convincing demonstration of a female biased sex ratio in pseudoarrhenotokous Phytoseiid mites under sib mating, which is the prediction of evolutionary theory if the sex ratio is under the control of the mother (Hamilton, 1967). They were further able to show that this control of the sex ratio by the mother is extremely precise (i.e. it conforms very closely to the sex ratio appropriate to the degree of inbreeding) (Nagelkerke and Sabelis, 1991; Sabelis and Nagelkerke, 1993). Similar facultative adjustment of the sex ratio has also been demonstrated in mealybugs (Homoptera: Pseudococcidae) which have the (pseudoarrhenotokous) lecanoid genetic system (Varndell and Godfray, 1996). This supports the circumstantial evidence for maternal control of inactivation that it is always the paternal chromosomes which are heterochromatised. The mechanism is unknown but requires that paternally and maternally derived chromosomes can be distinguished (i.e. there is some form of imprinting (Brown and Chandra, 1977; Solter, 1988)), and that the chromosome set identified as paternal can be selectively heterochromatised. These mechanisms allow a female to alter the offspring sex ratio whenever investment in one sex becomes more profitable.

In order for this to be an advantage, however, it must be harder for a female to take control of the sex ratio of her offspring in a diplodiploid system than in a haplodiploid

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[36] For a discussion of occurrences of chromosomal drive in nature see Trivers (1988).

one. Sex ratio manipulation by meiotic bias is rare in diplodiploids but has been recorded in psocids (book lice) (Mockford, 1971; Schneider, 1955), aphids (Hille Ris Lambers, 1966), some cladocerans (Fowler, 1909) and a variety of dipterans e.g. fungus gnats of the genus *Sciara* (Metz, 1926) and *Drosophila pseudoobscura* (Gershenson, 1928). The 'sex ratio' condition in *Drosophila pseudoobscura* causes the majority of Y chromosomes to degenerate leading to strongly female biased sex ratios, with only occasional sexually competent males (Policansky and Ellison, 1970). In *Drosophila paramelanica* there is destruction of Y bearing, male producing, sperm resulting in female biased sex ratios (Stalker, 1961). It seems likely that inbreeding is common in the natural populations of this fly in which this condition is prevalent and that the trait may be the result of selection for sex ratio control, however, such mechanisms must be far less precise than the control of sex ratio in haplodiploids and must incur a large cost in the production of sterile males. Precise control of the sex ratio does occur in aphids with cyclical parthenogenesis (Yamaguchi, 1985). Sex ratio manipulation by mothers may be possible in species in which the female is the heterogametic sex (butterflies, birds etc.) by biasing meiotic segregation in favour of one or other sex. Whilst this is a theoretical possibility there is no direct evidence of such a mechanism. Diplodiploids may manipulate the sex ratios by means of differential mortality. Trivers and Willard (1973) presented evidence for sex ratio control in various mammals in which mothers seem able to correlate the sex of their offspring with their own physical condition. The mechanism is likely to be selective abortion of embryos (O'Gara, 1969; Trivers, 1974). This mechanism is only feasible in organisms in which sex is determined at an early stage in ontogeny, before too much investment has occurred, keeping the cost of abortion low. Birds may control the sex ratio of their offspring by selective brood reduction (Howe, 1978) but the cost of this must be high since a great deal of resources will already have been invested at this point. This means that the expected payoffs for altering the brood sex ratio in this way must be large. Alexander and Sherman (1977) suggested that diplodiploid termites, which appear to be able to control the sex ratio of their reproductive offspring, may do so by controlling the proportion of each sex which develop as far as sexual maturity. In termites, as in mammals, no meiotic bias favouring one or other sex has ever been demonstrated and all differences can be accounted for by variation in mortality and development.[37]

The Inbreeding Hypotheses

Maternal son genetic systems are strongly correlated with chronic inbreeding (Ghiselin, 1974). This has led to a class of hypotheses which suggest that these systems have arisen as a response to this life cycle parameter. These hypotheses suggest that haplodiploidy is more likely to arise in inbred populations than in outbred ones because inbreeding lowers

[37] For recent reviews of sex ratio control see Werren (1987), Krackow (1995) and Godfray and Werren (1996). For a discussion of the effect of local mate competition on sex ratio control see Bull (1983).

the costs of haploid males relative to diploid males. They do not give an intrinsic advantage to uniparental systems but they eliminate many of the advantages diplodiploid systems usually have over haplodiploid systems in outbred species. There are four inbreeding hypotheses the inbreeding depression hypothesis, the heterozygote advantage hypothesis and two variable gamete hypotheses, the variable offspring hypothesis and the gamete competition hypothesis. The variable offspring hypothesis can account for all uniparental genetic systems but the gamete selection hypothesis can only account for the evolution of systems in which the uniparental offspring are male and the inbreeding depression hypothesis and the heterozygote advantage hypothesis can only account for haplodiploid systems.

A high level of inbreeding has been reported in many haplodiploid groups including thrips (Thysanoptera) (Lewis, 1973), scale insects (Coccoidea) (Beardsley and Gonzalez, 1975), parasitoid hymenoptera (Hamilton, 1967), beetles in the families Scolytidae (Entwhistle, 1964) and Micromalthidae (Scott, 1936) which live in highly inbred colonies in dead trees and rotting wood (Hamilton, 1978), and many mites including spider mites (Tetranychidae) (de Boer, 1985) and the arrhenotokous astigmatid parasite of earthworms *Histiostoma murchiei* (Histiostomatidae) (Oliver, 1962).

The Variable Gamete Hypotheses

Haploid individuals can produce only one gamete genotype. However, inbreeding abolishes the advantage diploids have over haploids due to their ability to produce an almost unlimited variety of gametes, since under inbreeding diploids will be homozygous at most loci and therefore will produce only a few different gamete genotypes. there are two variable gamete hypotheses, the variable offspring hypothesis and the gamete selection hypothesis.

The Variable Offspring Hypothesis

In the absence of polyembryony in which large numbers of offspring are produced from a single union of gametes, variable gametes generally result in variable offspring. Diploid males under inbreeding will have no advantage over haploids due to greater genetic variation in the offspring (Borgia, 1980).[38]

Hypotheses consistent only with systems in which males are the uniparental offspring

One of the variable gamete hypotheses, the gamete selection hypothesis will account for systems in which males are the uniparental offspring but is unlikely to be important in those in which the uniparental offspring are female.

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[38] Although this depends on the fitness distribution of the gamete genotypes. If one gamete genotype is much fitter than all the rest in terms of either gamete selection or selection on the zygote then most of the offspring will be derived from gametes with this genotype.

The Gamete Selection Hypothesis

Diploid males under inbreeding will have no advantage over haploids due to the opportunity for sperm competition. In systems in which females are the uniparental offspring the opportunity for gamete selection on the oocyte is lost but this is not a strong force and is therefore unlikely to have a large effect.

Hypotheses consistent only with haplodiploid systems

Two inbreeding hypotheses, the inbreeding depression hypothesis and the heterozygote hypothesis as well as the developmental rate hypothesis, and the two hemizygosity hypotheses, the deleterious allele hypothesis and the beneficial allele hypothesis (both of which can be formulated in terms of either individual selection or group selection) account for the evolution of haplodiploid systems. Since they all rely on one sex being haploid none of these hypotheses will account for germ line postfertilisation systems in which both sexes are diploid.

The Inbreeding Depression Hypothesis

Haplodiploids suffer less inbreeding depression than diplodiploids (Werren, 1993). Inbreeding populations are preadapted to the expression of deleterious recessive alleles due to the expression of these alleles in homozygotes in natural back crosses (Brown, 1964). Inbreeding diplodiploid populations are, therefore, likely to have far fewer deleterious alleles which would tend to prevent the evolution of haplodiploidy by decreasing the fitness of hemizygous haploids relative to that of heterozygous diploids in which such effects are masked. Haplodiploid populations are expected to suffer the same degree of inbreeding depression as a diplodiploid population with the same number of recessive deleterious mutations per capita when moving from an outbred to an inbred condition however, due greater selection efficiency, haplodiploid populations will have fewer recessive deleterious alleles per capita. This would not apply to germ line parahaploids since the effects are realised prior to gametogenesis. Since the effects of this advantage are mediated by hemizygosity in haploid individuals it would apply to any haplodiploid uniparental genetic system but not to systems with germ line genome loss.

Resistance to the deleterious effects of inbreeding depression would be of particular advantage to species characterised by frequent severe population crashes resulting in periods of very low effective population size and high levels of inbreeding. In such species any adaptation of the genetic system which would decrease the deleterious effects of inbreeding depression would increase the probability that the population would survive the population crash and is therefore likely to be favoured by group selection.

Since two thirds of all deleterious alleles in a haplodiploid population with a 1:1 sex ratio are found in a diploid individual a new recessive deleterious mutation can be expected to

last an average of three generations before it becomes expressed in the hemizygous state. Haplodiploid populations will therefore still retain some recessive deleterious alleles and therefore some degree of inbreeding depression in inbred populations although this will be less than for diplodiploid populations with the same degree of inbreeding. Hoy (1977) found only minimal effects of inbreeding in the pseudoarrhenotokous Phytoseiid mite *Metaseiulus occidentalis* as did Biemont and Bouletreau (1980) in the arrhenotokous wasp *Conothaspis boulardi*, however, inbreeding effects have been noted in a number of other haplodiploid species including monogonant rotifers of the genus *Asplanchna* (Birky, 1967).

The Heterozygote Advantage Hypothesis

Inbreeding increases the fitness of haploids relative to that of diploids since under inbreeding diploids are likely to be homozygous for most alleles and therefore, like haploids under either inbreeding or outbreeding, unable to benefit from any positive effects of heterozygosity (Borgia, 1980), i.e. inbreeding may make the evolution of haplodiploidy more likely by lowering the costs associated with it relative to diplodiploidy. As in the inbreeding depression hypothesis this would not apply to systems with germ line genome loss since most effects of natural selection are realised prior to gametogenesis.

The Developmental Rate Hypothesis

Elimination of DNA may lead to a faster mitotic cycle time (Cavalier-Smith, 1978) increasing the rate of development in the haploid sex. This will increase the number of matings that a member of the haploid sex will obtain during his lifetime and therefore there may be a selective advantage of haplodiploidy to the haploid sex. In genetic systems in which the uniparental parent is the opposite sex to the uniparental offspring there is a trade-off between the number of extra matings obtained and the loss of representation in haploid uniparental offspring. This will benefit the uniparental offspring in any haplodiploid genetic system in which the uniparental offspring can increase their reproductive success by increasing their developmental rate. This will be true as long as there is no adverse effect on mating success or fertility and lifetime fecundity is correlated with reproductive success. This is particularly likely to favour systems in which the uniparental parent and the uniparental offspring are the same sex since there will be no loss of representation in the haploid sex to offset the increase in developmental rate and these two forces may act synergistically to favour the evolution of such systems, i.e. there is no conflict between the sexes - the evolution of haplodiploidy is in the interests of both.

An increased rate of development may also facilitate the evolution of oedipal mating in species which disperse as unmated females and must initiate new populations by mating

with their own sons such as the arrhenotokous astigmatid mite *Histiostoma murchiei* (Histiostomatidae). Males of this species are haploid and reach sexual maturity in two days (Oliver, 1962), however this accelerated development is more likely to be due to loss of a stage in the life cycle than to haploidy. In such species a fast rate of development in the male decreases the costs to a female incurred in waiting for her sons to become sexually mature before she can mate with them and produce daughters[39]. This can only apply to arrhenotokes since only arrhenotokous females produce male offspring without mating. If the time taken between hatching and production of the first offspring is an important determinant of reproductive success in arrhenotokes then, in the absence of genetic and developmental constraints, arrhenotoky might be expected to be associated with other mechanisms for decreasing generation time such as paedogenesis.[40]

The Hemizygosity Hypotheses

The following two hypotheses are both based on the feature of haplodiploidy that one sex is haploid and therefore hemizygous for all loci. At any point in time, assuming a 1:1 sex ratio, one third of all alleles in a haplodiploid population will be in the hemizygous state. This proportion will be greater in populations in which the sex ratio is biased in favour of the haploid sex. Each of these hypotheses can be formulated in terms of either group selection or individual selection. Group selection hypotheses postulate that haplodiploidy is beneficial to the group rather than the individual and that there is selection between groups which vary in fitness according to the genetic systems of the individuals which they contain.

The Deleterious Allele Hypotheses

These hypotheses are based on the greater selection efficiency of haploids due to the expression of deleterious alleles in the hemizygous state. Haploid individuals have greater variance in fitness than diploid individuals. Sex differences in fitness variance may have important consequences for population genetics.

The Group Selectionist Deleterious Allele Hypothesis

This group selection hypothesis suggests that deleterious alleles are eliminated from a haplodiploid population faster than they would be from a diplodiploid population due to greater selection efficiency in haploids (Griffing, 1982). This results in a haplodiploid population with a lower genetic load which is therefore fitter than sympatric and parapatric diplodiploid populations. Selection between groups will therefore favour haplodiploid groups which will represent an ever increasing proportion of groups until all

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[39] But see Brown (1964).

[40] Arrhenotoky is associated with paedogenesis in the beetle *Micromalthus debilis* (Micromalthidae).

groups are haplodiploid. There are however problems with this hypothesis. Although haplodiploid populations have a lower genetic load than diplodiploid ones a greater proportion of this load is expressed per generation. It is not clear exactly how a decreased genetic load increases the fitness of a deme. It is only when a high degree of inbreeding occurs in a previously outbred population (e.g. due to population bottlenecking) that a haplodiploid deme will have an intrinsic advantage over a diplodiploid one load since the population with the higher genetic load can be expected to suffer more inbreeding depression. This hypothesis is therefore a restatement of the inbreeding depression hypothesis.

The Individual Selectionist Deleterious Allele Hypothesis

Goldstein (1994) has proposed an individual selectionist version of the deleterious allele hypothesis. This model is an extension of models designed to find parameters which would favour either haploidy or diploidy in both sexes[41]. Models with no sex differences in ploidy which assume that all gametes are formed by meiosis predict that deleterious alleles can confer an advantage to haploidy if selection against deleterious alleles is strong, expression of deleterious alleles in heterozygotes is low and there is tight linkage between the locus controlling ploidy and the loci at which deleterious alleles segregate (i.e. the recombination rate is low) (Bengtsson, 1992; Otto and Goldstein, 1992; Perrot *et al.*, 1991).

In Goldstein's model sexes may differ in ploidy (each sex can be either haploid or diploid) and under arrhenotoky spermatogenesis is mitotic. This model gave much the same results as models in which ploidy was always the same for both sexes and all gametes were formed by meiosis; arrhenotoky can successfully invade if selection against deleterious alleles is strong, expression of deleterious alleles in heterozygotes is low and the locus for arrhenotoky is tightly linked to loci at which deleterious alleles segregate (i.e. the recombination rate is low). There was however, a quantitative difference between the two types of model with a narrow window of parameter space allowing invasion of genes for arrhenotoky but not allowing for invasion of a gene for haploidy in both sexes (sex-independent haploidy). This is a consequence of the assumption that under arrhenotoky spermatogenesis is mitotic. This means that introduction of a gene for arrhenotoky is concomitant with a decrease in the amount of recombination in the population; a factor which itself predisposes to haploidy in this type of model since it tightens linkage between the genetic system locus an loci at which deleterious alleles segregate.

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[41] Crow (1965), Crow and Kimura (1988), Kondrashov and Crow (1991), Perrot *et al.* (1991), Perrot (1994), Goldstein (1992), Bengtsson (1992), Otto and Goldstein (1992), Otto (1994), Jenkins and Kirkpatrick (1994), Orr (1995).

Deleterious alleles promote haploidy when recombination rates are low because this causes linkage disequilibrium between genes for haploidy and non-deleterious alleles at loci at which deleterious alleles segregate. This is due to the greater selection efficiency against deleterious alleles in haploids. Assuming expression of deleterious alleles in heterozygous diploids is low, a greater proportion of the deleterious alleles present in haploids will be expressed than those present in diploids. If selection is on viability then individuals expressing the deleterious alleles will be less likely to survive to reproduce. This means that a greater proportion of the deleterious alleles present in haploids will be lost prior to reproduction than those in diploids. On average those haploids which do survive to reproduce will have fewer deleterious alleles than diploids so haploidy will be inherited together with fewer deleterious alleles than diploidy will and the mean fitness of haploids will be greater than that of diploids. This argument can easily be rephrased in terms of fecundity selection and holds true whether the gene for haploidy is expressed in both sexes or only in one. If the recombination rate is too high then the linkage disequilibrium breaks down and the advantage to diploids of masking deleterious alleles in the heterozygous condition outweighs the advantage haploids receive from greater selection efficiency and haploidy cannot invade i.e. there is a trade-off between these two forces, the outcome of which is determined by the recombination rate.

Since for the majority of parameter space favouring haploidy this model predicts that deleterious alleles would allow the invasion of either arrhenotoky or sex-independent haploidy it remains to be explained why arrhenotoky has arisen a number of times in the Metazoa whereas sex-independent haploidy has not. Sex-independent haploidy is quite common outside the Metazoa (Bell, 1994) but unknown within it; perhaps there are unique constraints on the evolution of sex-independent haploidy within the Metazoa. It seems unlikely that all of the independent origins of arrhenotoky took place in the narrow window of parameter space which, due to the absence of recombination in spermatogenesis in arrhenotokes, favours arrhenotoky but not sex-independent haploidy.

The Beneficial Allele Hypotheses

These hypotheses are based on the fitness advantages gained from expression of beneficial recessive alleles in the hemizygous state in the haploid individual.

The Group Selectionist Beneficial Allele Hypotheses

This group selection hypothesis supposes that haploidy allows the immediate expression of beneficial recessive alleles which arise in the population without the prior requirement that they must first occur together in the same diploid individual, leading to faster evolution of local adaptations in colonising species, such as shifts in host resistance etc. This is consistent with the observation that although haplodiploid species commonly exploit both diplodiploid and haplodiploid species as specialised parasites there is no

known example of a diplodiploid species exploiting a haplodiploid species in this way (Norton *et al.*, 1993).

The Individual Selectionist Beneficial Allele Hypotheses

The expression of beneficial recessive alleles would be of benefit to the individual expressing them as well as to the group as a whole, indeed if this were not so they could not be of benefit to the group since they would not increase in frequency in the group to a level at which they could increase the fitness of the entire group enough to influence intergroup selection. Since group selection is likely to be much slower than individual selection and cannot exert an influence until individual selection has already done so, it is not likely to be an important force in the evolution of haplodiploidy.

Hypotheses consistent only with prefertilisation systems

Hypotheses consistent only with prefertilisation systems are those based on the advantage to an individual of being able to produce offspring without the prior requirement for finding a mate. This is theoretically possible for either sex but due to the strong constraints on androgenesis it is much more likely that a female will be able to capitalise on these advantages than a male

The Facultative Asexuality Hypotheses

There is a class of hypotheses which suggest that arrhenotoky arose due to the ability of females to reproduce parthenogenetically when required. These hypotheses may account for the evolution of arrhenotoky but cannot account for pseudoarrhenotoky or pseudogamous arrhenotoky in which mating is obligatory (although pseudogamy may decrease the costs associated with finding a mate). One of these hypotheses, the population density hypothesis, is applicable to all prefertilisation systems including all types of parthenogenesis and, subject to constraints, androgenesis. Two facultative asexuality hypotheses, the Oedipal mating hypotheses can only account for arrhenotoky since they rely on a unique property of this system.

The Population Density Hypothesis

Arrhenotoky, but not pseudoarrhenotoky, confers an advantage to a female when the risk of failing to obtain a mate is high since in the absence of males an arrhenotokous female can produce male offspring by parthenogenesis. In the absence of genetic or developmental constraints on the evolution of arrhenotoky from pseudoarrhenotoky one would therefore expect pseudoarrhenotokous females to have a high probability of finding a mate. This would be a characteristic of species with high population densities and short range dispersal or in which dispersal occurs after mating, i.e. new habitat patches are colonised by inseminated adult females. These conditions would also be

likely to lead to a high degree of inbreeding which could also predispose to the evolution of haplodiploidy and these factors could well be acting together synergistically in species with these kinds of life histories and ecology. Species with low population densities, long range dispersal, dispersal prior to mating and strongly female biased sex ratios would be likely evolve rapidly into arrhenotokes.[42] This can only work for prefertilisation uniparental genetic systems, i.e. arrhenotoky or prefertilisation maternal daughter systems. Since in prefertilisation maternal daughter systems the parthenogenetically produced offspring would all be female this would be a less appropriate response to a low density of males than arrhenotoky in which the parthenogenetically produced offspring would all be male.

Hypotheses consistent only with arrhenotoky

Two facultative asexuality hypotheses, the Oedipal mating hypotheses (the reproductive success hypothesis and the colonisation hypothesis) can only account for arrhenotoky since they rely on a unique property of this system, the ability of virgin females to produce male offspring parthenogenetically.

The Oedipal Mating Hypotheses

These facultative parthenogen hypotheses suggests that the importance of facultative parthenogenesis lies in allowing uninseminated females to provide themselves with mates. Since these are facultative parthenogen hypotheses they cannot apply to pseudoarrhenotoky, however they may be able to account for the evolution of arrhenotoky. These hypotheses cannot account for any other uniparental genetic system than arrhenotoky since it is the only uniparental genetic systems in which unmated females can produce males. In prefertilisation maternal daughter systems new colonies can also be formed by unmated females but these will be effectively thelytokous until at least one male reaches the colony from elsewhere.

The Reproductive Success Hypothesis

This oedipal mating hypothesis suggests that in low population densities haploid males whose mothers were unfertilised (and therefore failed to find a mate) may have difficulties finding mates for themselves, however, if mother-son matings are possible these may be valuable to the females since they provide an opportunity to produce offspring whose reproductive success is not dependent on their ability to find a mate, i.e. oedipal mating ensures that a female's sons will be able to mate. There is evidence for oedipal mating in various parasitic haplodiploid insects, e.g. *Melittobia acasia* (a chalcid parasite of bees and wasps) (Balfour-Browne, 1922), *Cephalonomia quadridentata* (Van

[42] For a discussion of the relationship between genetic system and stage of dispersal see Mitchell (1970).

Emden, 1931) and the ambrosia beetle *Xyleborus compactus* (Scolytidae) (Entwhistle, 1964).

The Colonisation Hypothesis

This oedipal mating hypothesis suggests that in diplodiploidy and pseudoarrhenotoky a new habitat patch can only be colonised by a preinseminated female, or by a breeding pair but in arrhenotoky a new habitat patch can be colonised by a single uninseminated female who can parthenogenetically produce males with which to mate subsequently producing daughters for her sons to mate with and in this way founding a new colony (Gould, 1983). This is likely to be of particular importance in parasites in which the new habitat patch represents an uninfected host.

This system has been modelled by Adamson and Ludwig (1993) who predicted that colonising rates of greater than two parasites per host should favour the production of at least some dispersing males. The value of colonisation rate above which males would be expected to disperse is actually 2Wr where 1-Wr is the cost to a female of having to produce and mate with her son (including the time spent waiting for him to reach sexual maturity). Since the maximum value of Wr is 1 the maximum value of the colonisation rate is 2. This will be substantially lower if the cost is high. Oedipal mating is therefore only likely to be important in species with low rates of colonisation such as are expected when a parasite is switching to a new host species. Parasites capable of oedipal mating may therefore have lower host specificity and greater rates of adaptive radiation than those without.

In the arrhenotokous astigmatid mite *Histiostoma murchiei*, a parasite of the cocoons of earthworms described by Oliver (1962), new hosts are only ever infested by females in a preadult (i.e. preinsemination) stase called the hypopus. Males do not have a hypopodal stase and never leave the host in which they are born. Once a female gains access to a new host she must therefore produce unfertilised eggs giving rise to males with which she can mate before she can produce daughters which can in turn go on to infect further hosts. The cost to a female of waiting for her sons to develop before she can mate is reduced by males having a greatly accelerated life cycle and reach sexual maturity within two days. Oliver estimated a colonisation rate of 2.3 adult females per cocoon. The model of Adamson and Ludwig (1993) predicts that there should be at least some dispersing males, however, it is likely that in the past (when the population size of the parasite was lower shortly after it switched to its current host species) the colonisation rate was lower. Under these conditions there would have been a selective pressure on males to speed up their rate of development by omitting the hypopodal stase and thereby giving up the opportunity to disperse. As the population size rose and an increasing proportion of hosts became infected, the colonisation rate would have increased until it reached a level at which it would be profitable for males to disperse. However by this time the males had

lost this ability and the lack of a hypopus was acting as a constraint preventing the parasite from capitalising on the advantage to be gained from letting males disperse.

Another arrhenotokous mite, *Macrocheles muscadomestica* (Mesostigmata: Macrochelidae), a predator of early stages of development of house flies, disperse as uninseminated females phoretic on adult flies (Pereira and Castro, 1947). Oedipal mating may be frequent in many other Mesostigmata which disperse as deutonymphs (Norton *et al.*, 1993). de Boer (1985) suggested that most spider mite (family Tetranychidae) populations may have their origins in oedipal matings. Mitchell (1970) and Potter (1979) disagree, at least in terms of long range dispersal but Kennedy and Smitley (1985) suggest that there are two levels of dispersal with dispersal to new host plants effected by mated females and dispersal to new leaves of an already infested plant effected by uninseminated females unlikely to be able to find mates and hence relying on oedipal mating, however, there is very little documentary evidence to support this view. Male biased sex-ratios in arrhenotokous western flower thrips early in the season have been explained as a result of virgin females producing sons in the absence of males (Higgins and Myers, 1992).

Arrhenotokous pinworms (Nematoda: Oxyurida) live as adults in the posterior gut of their hosts and are transmitted by host larvae as eggs passed in the faeces (Adamson, 1989). Since all progeny disperse oedipal mating would not be expected. In some species however, females produce two different types of eggs; thin shelled eggs which hatch inside the host and do not disperse and thick shelled eggs arrested at the 2 or 4 cell stage which must pass to another host before they can continue their development. In *Gyrinicola batrachiensis* and *Tachygonetria vivipara* there is alternation of generations with females from thin shelled eggs producing only thick shelled eggs and vice versa (Adamson, 1981a; Adamson, 1981b; Adamson, 1981c; Adamson and Petter, 1983b). The sex ratio of the thick shelled eggs, i.e. the dispersing generation is strongly female biased and therefore oedipal mating may be important for colonisation of a new host.

In summary, oedipal mating may allow successful colonisation in arrhenotokous species in which only or predominantly unmated females disperse. However, this type of life history is restricted to only a few cases which has led Adamson and Ludwig (1993) to reject the hypothesis of Gould (1983) that such life histories are ancestral in haplodiploids.

Hypotheses consistent with pseudoarrhenotoky but not arrhenotoky

The Repair Hypothesis

If the risk of failing to obtain a mate is low, then pseudoarrhenotoky may confer an advantage over arrhenotoky by virtue of the opportunity for repair of the maternal genome in diploid male embryos prior to heterochromatisation, i.e. there may be a trade-

off between the risk of remaining unmated and the ability to repair somatic errors which may be fatal during the early stages of embryogenesis. This is more likely to be the case in germ line pseudoarrhenotoky since in somatic pseudoarrhenotoky the paternal genome is heterochromatised and therefore presumably unavailable for mismatch repair. This is a potential advantage of all postfertilisation uniparental genetic systems over their corresponding prefertilisation systems.[43]

CONCLUSIONS

The only class of hypotheses for the evolution of uniparental genetic systems which would appear to explain the puzzling lack of systems other than maternal sons are the oedipal mating hypotheses since the advantage of these hypotheses comes from the ability of a female to produce male offspring without mating. These hypotheses however cannot account for pseudoarrhenotoky since in this system mating must always occur. If oedipal mating are indeed responsible for the majority of the selective force giving rise to arrhenotoky then it is not likely that pseudoarrhenotoky is an intermediates in the evolution of arrhenotoky from ancestral diplodiploidy as has been suggested by some authors (e.g. Schrader and Hughes-Schrader (1931)). More likely is the possibility that arrhenotoky arose directly from biparentalism due to the advantage of oedipal mating and pseudoarrhenotoky either arose independently from biparentalism due to some other advantage or it arose from arrhenotoky due to some requirement for diploidy early in embryogenesis. The first of these possibilities however seems unlikely since groups with pseudoarrhenotoky are usually found in groups which also contain arrhenotoky suggesting that one is derived directly from the other.

Another alternative is that all postfertilisation systems arise from time to time in association with one or more of the other potential advantages suggested above but that in the case of maternal son postfertilisation systems these quickly evolve towards prefertilisation uniparentalism due to the advantage of facultative parthenogenesis whereas in other systems this shift towards postfertilisation systems does not occur either because it is not advantageous or due to constraints (e.g. the constraint on androgenesis in the case of paternal systems). Postfertilisation maternal daughter systems may also quickly evolve into prefertilisation systems due to the advantage of facultative parthenogenesis but in such systems males are likely to be lost quickly and the species become thelytokous. This would explain both the prevalence of arrhenotoky and thelytoky *and* the rarity of other prefertilisation systems.

If this hypothesis were true however, we would expect to find examples of postfertilisation paternal systems in nature but we do not. There are two potential reasons

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[43] For further discussion of DNA repair as an advantage of diploidy see Bernstein *et al.* (Bernstein, Hopf and Michod, 1988), Bernstein and Bernstein (1991), Avise (1993) and Michod and Gayley (1994) , Michod (1995).

for this, observational bias and cytoplasmic effects. Prefertilisation systems are much more likely to be detected than postfertilisation systems. This is because the identification of a postfertilisation system (particularly one in which the genome of the biparental parent is only lost just prior to gametogenesis) requires the observation of meiotic segregation bias whereas prefertilisation systems merely require the observation of offspring production by virgins. Once a prefertilisation system has been discovered it is likely that further work on the group will uncover the existence of postfertilisation systems in other species if they are present, however, in groups which do not have members with prefertilisation systems the existence of postfertilisation systems may go unnoticed. Alternatively the lack of postfertilisation paternal systems may be due to maternally inherited cytoplasmic factors which will tend to prevent the elimination of the maternal genome. Since males contribute no such factors to the offspring they are less able to prevent the elimination of the genome which they contribute to their offspring and this leads to the evolution of postfertilisation genetic systems in which the female is the uniparental parent and the subsequent evolution of corresponding prefertilisation systems. These two ideas are discussed in greater detail in chapter 7.

We are a long way from resolving these issues at present. A thorough investigation of the genetic systems of a wide range of metazoan taxa coupled with a rigorous phylogenetic analysis of groups containing both prefertilisation and postfertilisation systems is needed before it will be possible to say with any degree of certainty which of the competing hypotheses for the evolution of uniparental genetic systems is of the greatest importance. If we rely on the incidental observations of genetic systems arising from other research programs as the sole source of our data (as has been the approach to this problem in the past) then we are likely to be confounded by the problems of observational bias.

Chapter Three

Uniparental Genetic Systems and the Evolution of Haplodiploidy. III. Phylogeny

Comparative biologists may understandably feel frustrated upon being told that they need to know the phylogenies of their groups in detail, when this is not something they had much interest in knowing. Nevertheless, phylogenies are fundamental to comparative biology; there is no doing it without taking them into account.

Joe Felsenstein, 1985b

SUMMARY

In 1931 Schrader and Hughes-Schrader suggested that arrhenotoky could arise through a succession of stages involving pseudoarrhenotokous systems such as those found in many scale insects (Homoptera: Coccoidea). In 1970 Hartl and Brown pointed out that if this was true then these intermediate pseudoarrhenotokous forms should also be found in other haplodiploid groups. Since at that time no other such examples were known they took this to be evidence that parahaploidy was *not* a necessary intermediate step in the evolution of arrhenotoky. More recently, however, pseudoarrhenotoky has been demonstrated in a number of species of mite, and one species of beetle, closely related to arrhenotokes. Here I re-examine the hypothesis of Schrader and Hughes-Schrader in the light of more recent evidence and conclude that there is no reason to reject this hypothesis. I then go on to examine in more detail whether current understanding of the phylogeny of genetic systems in the mites can be used to test this hypothesis.

INTRODUCTION

A great deal has been written about the evolutionary consequences of haplodiploidy, particularly in connection with sociality[44], however, comparatively little attention has been paid to the origins of this phenomenon. The reasons for this are at least twofold. Firstly, and perhaps most importantly, the origins of haplodiploidy lie in the remote past whereas its consequences can easily be observed in living organisms. Secondly, although haplodiploidy has arisen independently a number of times in widely differing metazoan taxa most of the research on this topic has been directed towards just one of these, the Hymenoptera. This large and ancient group of insects, with the exception of a few secondary asexuals, is universally haplodiploid and its phylogenetic position within the insects is poorly understood. This means that whilst the Hymenoptera may be an

[44] See for example Hamilton (1996) and references therein.

appropriate group in which to study the consequences of haplodiploidy it is not so useful for illuminating its origins.

Recently more attention has been paid to other groups of arthropods such as the scale insects (Homoptera: Coccoidea) and the mites (Arachnida: Acari), although almost exclusively to economically important pests such as the spider mites (Tetranychidae) and their natural enemies, the Phytoseiidae. This research is primarily directed towards the improvement of the biological control of these species. The mites are much a more promising group in which to investigate the evolution of haplodiploidy since they contain several independent origins of this genetic system (Heinemann and Hughes, 1969). Whilst knowledge of the genetic systems of mites is very far from complete, and nowhere near as detailed as that of the Hymenoptera, it has reached a state at which the historical sequence of genetic system evolution can begin to be meaningfully discussed. As new information comes to light this can be used to test and refine these first tentative hypotheses. The other component required for such a discussion is a knowledge of the phylogeny of the group and it is with this that any such research program must begin.

HAPLODIPLOIDY

There has been some confusion over the nomenclature of haplodiploidy. Haplodiploidy refers to a phenomenon in which males are haploid whilst females are diploid[45]. This is common to a number of different genetic systems. Some authors, however, have used the term haplodiploidy to refer to a *particular* genetic system in which males arise from unfertilised eggs whilst females arise from fertilised eggs. This phenomenon should not be referred to as haplodiploidy but as arrhenotoky. The reason for this is that although they are intimately connected haplodiploidy and arrhenotoky are distinct phenomena and can, indeed do, occur independently (table 3.1).

Table 3.1: The Relationship Between Karyotype and Genetic System

HAPLODIPLOIDY	ARRHENOTOKY	
+	-	**PSEUDOARRHENOTOKY**
-	+	**DIPLOID ARRHENOTOKY**

Haplodiploidy can occur in the absence of arrhenotoky if a biparentally produced male eliminates half of the genetic complement it has received from its parents early in embryogenesis. A genetic system in which the genome of one parent is eliminated in at least some of the offspring prior to gametogenesis is known as postfertilisation

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[45] Strictly speaking haplodiploidy refers to all systems in which individuals of one sex are haploid whilst those of the other are diploid (haplodiploidy *sensu lato*). Since in all known cases it is the males which are the haploid sex the remainder of this chapter will use the term haplodiploidy to refer to systems in which males are haploid whilst females are diploid (haplodiploidy *sensu stricto*).

uniparentalism or parahaploidy. If the *paternal* genome is eliminated in *male* offspring then this is known as pseudoarrhenotoky or paternal genome loss[46]. Pseudoarrhenotoky occurs in a number of taxa including scale insects and mites. Arrhenotoky can occur without haplodiploidy if a uniparentally produced male becomes diploid through fusion of the first two haploid cleavage nuclei. This phenomenon is referred to as diploid arrhenotoky and occurs in some scale insects (Nur, 1972; Phillips, 1965).

Male haploidy could therefore be due to a number of different genetic systems:

- **Arrhenotoky** in which case males are haploid because they do not receive a genome from their father.
- **Pseudoarrhenotoky** in which case males are haploid because although they receive a genome from their father this genome is lost early in embryogenesis.
- **Androgenesis** in which case males are haploid because they do not receive a genome from their mother.
- **Maternal genome loss** in which case males are haploid because although they receive a genome from their mother this genome is lost early in embryogenesis.
- **Other systems** - there is no requirement that the genome that is lost come from one parent so haploidy in males could be due to any combination of the above phenomena in which some chromosomal material of paternal origin and some of maternal origin are lost so that the remaining material constitutes a haploid genome.

In all cases in which the genetic system underlying haplodiploidy has been identified it has proven to be either arrhenotoky or pseudoarrhenotoky and therefore the remainder of this chapter will focus on these two genetic systems.

Arrhenotoky

Arrhenotoky is thought to be characteristic of one entire order of nematode worms: the pinworms (order Oxyurida) (Adamson, 1989). There have been at least five independent origins of arrhenotoky within the mites (class Acari) (Norton *et al.*, 1993). There have been at least two independent origins of arrhenotoky within the scale insects (superfamily Coccoidea); haplodiploid arrhenotoky in the giant scales (family Margodidae) (Hughes-Schrader, 1948) and diploid arrhenotoky in the soft scales (family Coccidae) (Nur, 1972; Phillips, 1965). Arrhenotoky is thought to be characteristic of two entire orders of insects; the thrips (Thysanoptera) (Davidson and Bald, 1931; Risler and Kempter, 1962; Shull,

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[46] Although strictly the term paternal genome loss could also apply to cases in which the paternal genome is lost in female offspring. Haplodiploidy could also occur in the absence of arrhenotoky due to *maternal* genome loss or to the loss of some combination of paternally and maternally derived material together constituting one complete genome, however, such systems are unknown in nature.

1914; Shull, 1917) and the Hymenoptera (Crozier, 1975). There appear to have been three independent origins of arrhenotoky in the beetles: one in the primitive beetle *Micromalthus debilis* (Scott, 1936; Scott, 1941) and two independent origins in bark beetles of the family Scolytidae; one in the tribe Xyleborini and another on the tribe Dryocetini (Kirkendall, 1993). Arrhenotoky is not just an esoteric curiosity. It has evolved independently at least 13 times in a number of different taxa (table 3.2) and represents the genetic system of at least 10% of all named species of animal (Gould, 1983).

Table 3.2: The Taxonomic Distribution of Arrhenotoky

GROUP	MINIMUM NUMBER OF INDEPENDENT ORIGINS
Pinworms	1
Mites	5
Scale Insects	2
Thrips	1
Hymenoptera	1
Beetles	3
TOTAL	**13**

Pseudoarrhenotoky

Pseudoarrhenotoky can be defined as any genetic system in which males are produced biparentally and receive a genome from their father but eliminate this at some point prior to spermatogenesis so that they do not pass it on to their own offspring. Since the elimination of the paternal genome can occur at any point in the ontogeny of the male pseudoarrhenotokous right up until just before spermatogenesis, males may be either haploid or diploid. Haplodiploidy therefore only occurs in pseudoarrhenotokes in which the paternal genome is eliminated early in embryogenesis.

All of the groups which contain pseudoarrhenotokes are ones which also contain arrhenotokes (table 3.3). This suggests that the two systems are in some way related. The mealy bugs, which constitute a family of scale insects called Pseudococcidae, have a diplodiploid pseudoarrhenotokous genetic system called the Lecanoid (or L) system (Hughes-Schrader, 1948; Schrader, 1923) in which the chromosomes of paternal origin are heterochromatised in male embryos (Brown and Nelson-Rees, 1961). These heterochromatic chromosomes are subsequently destroyed following prophase I of spermatogenesis (Schrader, 1929). Felted scales (family Eriococcidae) have a similar system called the Comstockiella (or C) system which is identical to the Lecanoid system except that the heterochromatised chromosomes are destroyed just prior to prophase I of spermatogenesis (Brown, 1963). Armoured scales (family Diaspididae) have a

haplodiploid pseudoarrhenotokous genetic system called the Diaspidid (or D) system in which the chromosomes of paternal origin are eliminated early in cleavage at about the same time that heterochromatisation occurs in the L and C systems (Brown and Bennett, 1957). Recent phylogenetic analyses of the scale insects suggest that the D system has evolved several times from the C system which in turn has evolved from the L system which along with haplodiploid arrhenotoky arose from an ancestral XX/XO sex chromosome system with heterogametic males. Diploid arrhenotoky may have arisen from either the L or C systems (Nur, 1980). Diplodiploid pseudoarrhenotoky has been identified in a scolytid beetle: the Coffee Berry Borer (*Hypothenemus hampei*) (Borsa and Kjellberg, 1996; Brun *et al.*, 1995). In addition to these examples of arrhenotoky and pseudoarrhenotoky there are also two important groups in which haplodiploidy has been identified but in which the precise nature of the underlying genetic system is still unknown. These are the monogonant rotifers and the whiteflies (superfamily Aleyrodoidea).

Table 3.3: Taxonomic Distribution of Pseudoarrhenotoky

GROUP	MINIMUM NUMBER OF INDEPENDENT ORIGINS
Mites	1
Scale Insects	1
Beetles	1
TOTAL	**3**

The Evolutionary Relationship Between Arrhenotoky and Pseudoarrhenotoky: The Hypothesis of Schrader and Hughes-Schrader

In 1931, Schrader and Hughes-Schrader suggested that arrhenotoky did not arise directly from a diplodiploid ancestor but rather that it evolved through a series of intermediate steps involving pseudoarrhenotokous systems:

> *"The historical hypotheses of haploidy hold in common the idea that the process of sex determination in haploid individuals differs from the more usual type only in some simple or perhaps single respect. It may therefore be pertinent to suggest that the differences between the two types may well be more far-reaching and that they have not been attained in a single step....*
>
> *Since species with haploid males have undoubtedly been derived from species in which they are diploid, certain changes must have occurred to make viability in the haploid state possible. It must also be recognized that the production of haploid individuals may not have been the result of a single accident of development .... but may represent the culmination of a whole series of minor changes. The latter possibility we believe to be exemplified by certain species of coccids....*
>
> *Within the group of the coccids are represented both beginning and end stages in the establishment of haploidy .... The expectation of finding species, within this group, representative of intermediate stages does not, therefore,*

seem entirely unjustified .... the ability to develop and to survive in the haploid condition is in process of acquisition by the male sex, not, as might at first be expected, by the sudden or accidental parthenogenetic development of a mature egg, but by a series of changes in the chromosomal conditions of the diploid male themselves .... The basic phenomenon .... is a gradual degeneration of one haploid set of chromosomes in the male; the process is reflected chiefly in the behaviour of the chromosomes during meiosis, but in its later stages the soma is also affected."

Franz Schrader and Sally Hughes-Schrader, 1931

According to this view there are three steps in the evolution of arrhenotoky from a zygogenetic diplodiploid ancestor (table 3.4). The first involves a switch in genetic system from zygogenesis to pseudoarrhenotoky, the second a switch in karyotype from diplodiploidy to haplodiploidy and the third a switch in genetic system from pseudoarrhenotoky to arrhenotoky (table 4). This means that there are four different genetic systems in this sequence. Stage one is ancestral diplodiploid zygogenesis, stage two, diplodiploid pseudoarrhenotoky, stage three, haplodiploid pseudoarrhenotoky and stage four haplodiploid arrhenotoky. Diploid arrhenotoky is presumably a further modification of haplodiploid arrhenotoky in which there has been a reversal in karyotype to diplodiploidy. This represents stage five. In the scale insects all five of these stages are represented. The mites contain known representatives of stages 1, 3 and 4 and the scolytid beetles, 1, 2 and 4. In both mites and scolytid beetles genetic systems are known in only a relatively small number of species and further investigation may well turn up representatives of the missing systems. In particular diplodiploid pseudoarrhenotoky may be mistaken for pseudogamy (or even zygogenesis) and haplodiploid pseudoarrhenotoky for arrhenotoky unless marker studies are used specifically to search for these systems.

Table 3.4: A Summary of the Hypothesis of Schrader and Hughes-Schrader

	KARYOTYPE	GENETIC SYSTEM
Stage 1	diplodiploidy	zygogenesis
Stage 2	diplodiploidy	pseudoarrhenotoky
Stage 3	haplodiploidy	pseudoarrhenotoky
Stage 4	haplodiploidy	arrhenotoky
Stage 5	diplodiploidy	arrhenotoky

The hypothesis of Schrader and Hughes-Schrader was largely ignored until 1970 when Hartl and Brown criticised it on three counts and the hypothesis has been largely ignored since. However these three criticisms although valid at the time that they were made have become obsolete in the light of more recent research and there is now some weight of evidence in favour of the hypothesis of Schrader and Hughes-Schrader. This therefore seems to be a good time to re-examine this hypothesis.

A Re-evaluation of Hartl and Brown's Criticisms of Schrader and Hughes-Schrader

Hart and Brown (1970) made three criticisms of the hypothesis of Schrader and Hughes-Schrader:

> *"Schrader and Hughes-Schrader (1931) have proposed that the haplodiploid iceryine coccids derived from progenitors with a parahaploid chromosome system not unlike that found in some of the modern coccids in which the paternal set of chromosomes becomes heterochromatic during the development of males. There are three reasons for judging this hypothesis insufficient: First, it fails to account for the taxonomic relationships among the coccids .... secondly, a thorough search of the evolutionary series of coccids in which heterochromatisation is found has revealed no evidence that this type of parahaploidy can lead to male haploidy .... and, thirdly, the hypothesis would require postulating evolutionary intermediates for the rotifers, mites, hymenopterans, etc., at least one of which might be expected to be extant today, but parahaploidy is unknown in these forms."*
>
> *Daniel L. Hartl and Spencer W. Brown, 1970*

I will now examine each of these criticisms to see whether they still hold true in the light of a more modern understanding of genetic systems.

Criticism #1: ***"it fails to account for the taxonomic relationships among the coccids"***

This is still partly true but whilst haplodiploid arrhenotoky and pseudoarrhenotoky seem to have independent origins from ancestral diplodiploid zygogenesis it does appear that diploid arrhenotoky has arisen from diplodiploid pseudoarrhenotoky (Nur, 1980). Such a scheme however postulates a number of missing intermediates.

Criticism #2: ***"a thorough search of the evolutionary series of coccids in which heterochromatisation is found has revealed no evidence that this type of parahaploidy can lead to male haploidy"***

Parahaploidy does indeed lead to male haploidy in the Diaspidid system of the armoured scales (family Diaspididae).

Criticism #3: ***"the hypothesis would require postulating evolutionary intermediates for the rotifers, mites, hymenopterans, etc., at least one of which might be expected to be extant today, but parahaploidy is unknown in these forms."***

Parahaploidy has been reported in a number of other haplodiploid taxa including mites and bark beetles and there is even some circumstantial evidence that parahaploid systems may be ancestral in the hymenoptera (see below). The fact that all known examples of pseudoarrhenotoky occur in groups which also contain arrhenotoky ~~is~~ suggests that they are phylogenetically related.

Evidence in Support of Schrader and Hughes-Schrader

There are a number of lines of evidence in favour of the hypothesis of Schrader and Hughes-Schrader:

- The concurrence of arrhenotoky and pseudoarrhenotoky is highly suggestive that the two are somehow evolutionarily related. The little we know about the phylogeny of these groups at least does not rule out the hypothesis.

- If Schrader and Hughes-Schrader are correct then we might expect to find states intermediate between the those outlined above. One possible example is represented by the arrhenotokous mites *Geolaelaps aculifer* and *Stratiolaelaps miles* which have a heterochromatic chromosome arm which has been interpreted as the vestiges of the heterochromatised complement of a pseudoarrhenotokous ancestor (DeJong *et al.*, 1981). Since these mites belong to a group which contains both haplodiploid pseudoarrhenotokes (stage 3) and haplodiploid arrhenotokes (stage 4). they may represent an intermediate between these two stages.

- Paternal genome loss can be induced by intracellular parasites (*Wolbachia*) in the solitary wasp *Nasonia vitripennis* (Hunter *et al.*, 1993; Reed and Werren, 1995; Werren *et al.*, 1995). The fact that this can be achieved may indicate that PGL has had some role in the normal genetic system of an ancestor of these hymenopterans.

In conclusion, current evidence does not provide any reason to reject the hypothesis of Schrader and Hughes-Schrader. On the contrary this hypothesis is a potentially important one which should be tested empirically. The most relevant test of the hypothesis would be a phylogeny of these genetic systems in those groups which contain, zygogenetic, pseudoarrhenotokous and arrhenotokous members. One such group is the mites but is our current understanding of the phylogeny of genetic systems in the mites sufficient to test this hypothesis? The next section of this review tries to answer this question.

HAPLODIPLOIDY IN THE MITES AND TICKS

The Acari (the mites and ticks) are divided into two distinct groups which may have independent origins within the arachnids[47], these groups are the Acariformes and the Parasitiformes (figure 3.1).

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[47] The Acari were first split into two groups by (the Actinochitinosi and the Anactinochitinosi) Grandjean (1935) on the basis of the optical properties of the setae, however, he considered the Acari as a whole to be monophyletic. Andre and Lamy (1937) were the first to suggest that the Acari are diphyletic. They considered the sister taxon of the Parasitiformes to be the Opiliones. Vizthum (1940 *et seq.*) also considered the Acari to be diphyletic but suggested that the sister taxon of the Parasitiformes is the Ricinulei, based on sharing of a hexapodal larva and a pair of lateral prosomal stigmata. Other proponents of a diphyletic Acari have been Zachvatkin (1952) and van der Hammen (1977; 1979; 1989). This literature has been reviewed by Lindquist (1984).

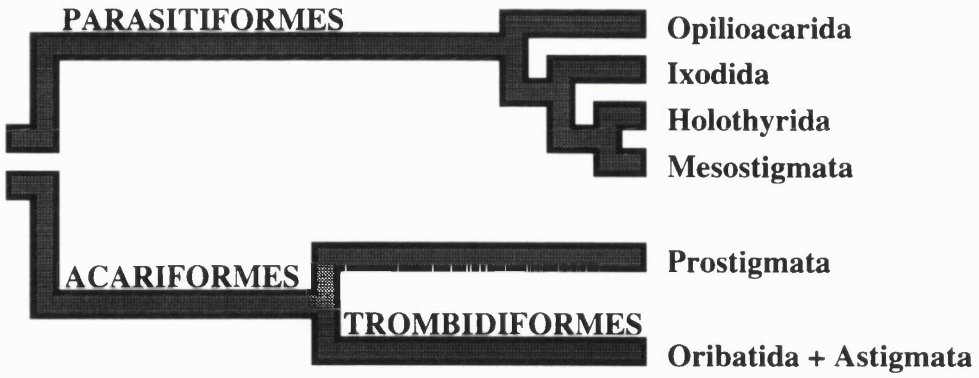

Figure 3.1: The Phylogeny of the Acari
After Norton *et al.* (1993), figure 1.1, p.10

Acariformes

The Acariform mites are divided into three artificial groups; the Endeostigmata, the Prostigmata and the Sarcoptiformes *sensu stricto*. The Prostigmata and the Sarcoptiformes *sensu stricto* are likely to be monophyletic, however, although previously believed to be a monophyletic group (e.g. Krantz (1978)), the Endeostigmata, which is made up of ten families of early derivative acariform mites, is now believed to be a paraphyletic group from which the Prostigmata and Sarcoptiformes *sensu stricto* are presumed to have independent origins (OConnor, 1984). The entire order can be divided into two monophyletic groups; the Trombidiformes and the Sarcoptiformes *sensu lato*. The Trombidiformes contains the Prostigmata and two families of the Endeostigmata[48] whilst the Sarcoptiformes *sensu lato* contains the Sarcoptiformes *sensu stricto* and the remaining eight families of the Endeostigmata[49] (Kethley in Norton *et al.* (1993)) (figure 3.2). Genetic system data is sparse but haplodiploid genetic systems have been identified in both the Prostigmata and the Sarcoptiformes *sensu stricto*[50]. The precise position of the Sarcoptiformes *sensu stricto* within the Sarcoptiformes *sensu lato* is unclear[51].

Prostigmata

The Prostigmata contains about 125 families of which diplodiploidy has been established as the sole genetic system of 13 and haplodiploidy the sole genetic system of 20. Of the

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[48] Sphaerolichidae (the putative sister group of the Prostigmata) and Lordalycidae

[49] Nanorchestidae, Bimichaeliidae, Nematalycidae, Proteonematalycidae, Micropsammidae, Oehserchestidae, Grandjeanicidae, Terpnacaridae and Alicorhagiidae.

[50] There are no data on the karyotypes or genetic systems of any species of Endeostigmata, however, Norton *et al.* (1993) predict that they maintain plesiotypic diplodiploidy and suggest candidate taxa for testing this hypothesis. Both the Prostigmata and the Sarcoptiformes *sensu stricto* contain both diplodiploid and arrhenotokous species which implies that there must have been at least one independent origin of arrhenotoky in each of theses groups. To date pseudoarrhenotoky has not yet been reported in any acariform mite.

[51] The putative sister group of the Sarcoptiformes *sensu stricto* has been interpreted variously as Terpnacaridae (Kethley, 1990) and Alicorhagiidae (OConnor, 1984)).

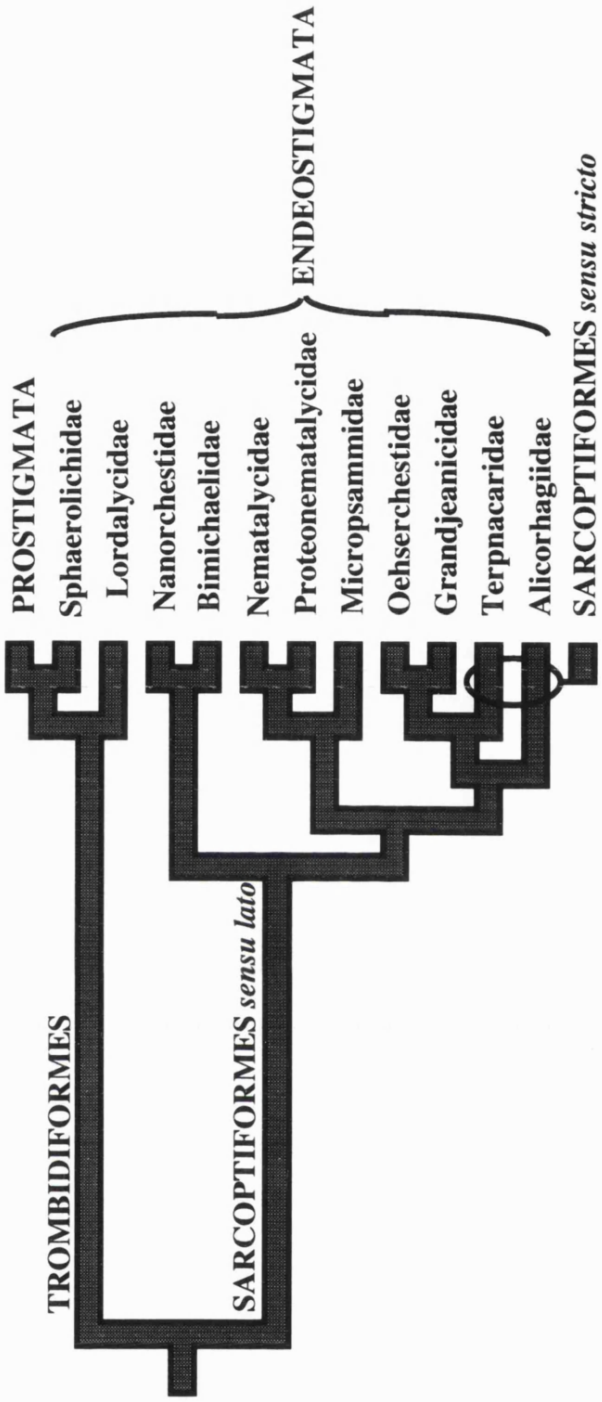

Figure 3.2: The Phylogeny of the Acariformes
After Norton *et al.* (1993), figure 1.3, p.22

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many species with haplodiploidy, arrhenotoky has been demonstrated in about half whilst in the remainder the exact nature of the genetic system is still unknown.

The Prostigmata can be divided into three monophyletic groups; the Anystina, the Eupodina and the Eleutherengona of which the Anystina and the Eupodina together form a monophyletic clade (Norton *et al.*, 1993). All reports of karyotypes in the Anystina are

of diplodiploidy[52] whereas all reports of karyotypes in the Eupodina[53] and Eleutherengona[54] are of haplodiploidy. Since diplodiploidy is assumed to be plesiotypic

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[52] The Anystina consists of the Parasitengona and four other families; the Anystidae, the Teneriffiidae, the Caeculidae and the Adamystidae. 33 species in 13 families of the Parasitengona have been shown to be diplodiploid (*Allothrombium fuliginosum* (2n = 24 although there is one report of 2n = 12 (Bottazzi cited in Oliver (1977)) and two species of *Sericothrombium* (2n = 18 and 22) (Trombidiidae), one species of *Erythraeus* (2n = 16) (Erythraeidae), two species of *Hydrachna* (2n = 12) (Hydrachnidae), *Thyas dirempta* (2n = 18) and two species of *Hydryphantes* (2n = 10) (Hydryphantidae), *Frontipoda musculus* (2n = 18) and two species of *Lebertia* (2n = 16 and 18) (Lebertiidae), two species of *Limnesia* (2n = 18) (Limnesiidae), *Hygrobates calliger* (2n = 14) (Hygrobatidae), *Unionicola crassipes* (2n = 18) and *Neumania vernalis* (2n = 4) (Unionicolidae), four species of *Piona* (2n = 8, 20 and 22) (Pionidae) and five species of *Arrhenurus* (2n = 20 and 26) (Arrhenuridae) (Sokolov, 1954); *Hydrodroma despiciens* (2n = 6) (Hydrodromidae) and two species of *Eylais* (2n = 4 and 6) (Erythraeidae) (Keyl, 1957; Sokolov, 1954); *Limnochares aquatica* (2n = 6) (Erythraeidae) (Sokolov, 1962; Sokolov, 1954) and *Leptotrombidium arenicola* (2n = 28), *L. deliense* (2n = 14) and *L. fletcheri* (2n= 14) (Trombiculidae) (Shirai *et al.*, 1984). The genetic systems of the other members of the Anystina are unknown.

[53] The Eupodina consists of the Labidostommatidae, the Eupodoidea, the Tydeoidea, the Eriophyoidea, the Bdelloidea and the Halacaroidea. ((Kethley (1982; 1990) does not consider the Bdelloidea or the Halacaroidea to be monophyletic groups but considers them together to form a monophyletic clade.) Male haploidy has been identified in two species of Eupodoidea (one species of *Eupodes* (n = 9) and one species of *Linopodes* (n = 9) (Eupodidae)(Sokolov, 1954) in which male haploidy was assumed on the basis of a chromosome counts of 9 which cannot be a diploid number unless males are XO; a system of sex determination unknown in the Prostigmata), two species of Tydeoidea (*Tydeus caudatus* (n = 2) (Tydedidae) (Helle and Wysoki, 1983) and *Riccardiella limacum* (n = 5) (Ereynetidae) (Helle *et al.*, 1984)), seven species of Eriophyoidea (all in the family Eriophyidae) (*Phyllocoptruta oleivora* (n = 2) (Swirski and Amitai, 1959); *Aceria sheldoni* (n=2) (Sternlicht and Goldenberg, 1971) and *Aculops tetanothrix* (n = 2), *Aculus persicae* (n = 2), *Aculus schlechtendali* (n = 2), *Artacris macrorhynchus* (n = 2) and *Phytopus tiliae* (n = 2) (Helle and Wysoki, 1983)) and one species of Bdelloidea (*Cunaxa capreolus* (n=11) (Cunaxidae) (Helle *et al.*, 1984)). The nature of the genetic system in the Labidostommatidae remains unknown. Arrhenotoky has been established as the genetic system in one species of Tydeoidea (*Homeopronematus anconai* (Tydedidae) (Knop and Hoy, 1983)) and nine species of Eriophyoidea (all in the family Eriophyidae) (*Aculops fockeui* (Oldfield, 1988; Putman, 1939); *Aculops lyucopersici* (Bailey and Keifer, 1943); *Aculops pelekassi* (Burditt, Reed and Crittenden, 1963); *Aculops cornatus* (Oldfield, Hobza and Wilson, 1970); *Phyllocoptruta oleivora* (Helle and Wysoki, 1983; Oldfield *et al.*, 1970)); *Eriophyes laevis* (Schevtchenko in Oliver (1971))); *Aceria sheldoni* (Helle and Wysoki, 1983); *Aculus schlechtendali* and *Epitrimerus pyri* (Oldfield, 1988))).

[54] The Eleutherengona consists of the Tetranychoidea, Cheyletoidea, Raphignathoidea, Pterygosomatoidea. Pomerantzioidea, Pseudocheylidae, Heterostigmata, Stigmocheylidae and Paratydeidae. Male haploidy has been identified in many species of Tetranychoidea (many species in the family Tetranychidae (Gutierrez, Bolland and Helle, 1979) and 20 species in the family Tenuipalpidae (*Brevipalpus russulus* (n = 2) (Pijnacker *et al.*, 1980); *Brevipalpus pulcher* (n = 2) and *Cenopalpus lanceolatisetae* (n = 2) (Helle, Bolland and Heitmans, 1980); ten species of *Brevipalpus* (n = 2) (Bolland and Helle, 1981; Helle *et al.*, 1980); *Aegyptobia ephedrae* (n = 2), *Brevipalpus spinosus* (n = 2), *Dolichotetranychus summersi* (n = 2), *Obuloides* sp. (n = 3), *Raoiella indica* (n = 2) and *Tenuipalpoides acaciae* (n = 3) (Bolland and Helle, 1981) and *Aegyptobia* sp. (n = 2) (Helle and Wysoki, 1983))), eight species of Cheyletoidea (*Harpyrhynchus brevis* (n = 2) (Harpyrhynchidae) (Oliver and Nelson, 1967); *Harpyrhynchus novoplumaris* (n = 2) (Harpyrhynchidae) (Moss, Oliver and Nelson, 1968); *Syringophiloidus minor* (n = 3) (Syringophilidae) (Casto, 1974); *Cheyletus malaccensis* (n = 2) (Cheyletidae) (Helle *et al.*, 1984; Regev, 1974); *Demodex caprae* (n = 2) (Demodicidae) (Lebel and Desch, 1979) and *Cheletogenes ornatus* (n = 2), *Acaropsellina docta* (n = 5) and *Nodele simplex* (n = 2) (Cheyletidae) (Helle *et al.*, 1984)), nine species of Raphignathoidea (*Neophyllobius elegans* (n = 11) (Camerobiidae) (Gutierrez *et al.*, 1979) ; *Neophyllobius aesculi* (n = 11) (Camerobiidae), *Agistemus exsertus* (n = 3) and *A. tranatalensis* (n=3) (Stigmaeidae) and *Saniosulus nudus* (n = 3) (Eupalopsellidae) (Helle *et al.*, 1984) and *Agistemus camerounensis* (n = 2) and *A. sanctiluciae* (n = 2) (Stigmaeidae), *Eupalopsellus brevipalpus* (n = 4) and *E. olearius* (n = 3) (Eupalopsellidae) (Bolland and Ueckermann, 1984)) and nine species of Heterostigmata (*Pyemotes ventricosus* (n = 3) (Pyemotidae) (Patau, 1936); *Siteroptes graminum* (n = 3) (Pygmephoridae) (Cooper, 1937) *Polyphagotarsonemus latus* (n = 2) (Tarsonemidae) (Gadd, 1946; Karl, 1965b; Nuciflora, 1963); *Phytonemus pallidus* (n = 2) (Tarsonemidae) (Karl, 1965a); one species of *Tarsonemus* (n = 2) (Tarsonemidae) (Helle *et al.*, 1984) and *Pyemotes tritici* (n = 3) (Pyemotidae), *Pediculaster flechtmanni* (n = 3), *Pediculaster mesembrinae* (n = 3) and *Siteroptes reniformis* (n = 3) (Pygmephoridae) (Kaliszewski in Norton *et al.* (1993)). The nature of the genetic system in the remaining members of the Eleutherengona is unknown. Arrhenotoky has been established in many species in the Tetranychoidea; many species in the family Tetranychidae (Helle and Pijnacker, 1985) and three species in the family Tenuipalpidae (*Brevipalpus pulcher* (Zaher, Soliman and El-Safi, 1974), *B. russulus* (Pijnacker *et al.*, 1980) and *B. spinosus* (Bolland and Helle, 1981); despite the assumption that arrhenotoky is the genetic system of all nonthelytokous members of the Tenuipalpidae for which male haploidy has been established (Bolland and Helle, 1981; Helle *et al.*, 1980) it has only been proven in these three species), one species of Pterygosomatoidea (*Geckobiella texana* (Pterygosomatidae) (Goodwin, 1954) and 20 species of Heterostigmata (*Pyemotes ventricosus* (Pyemotidae)(Patau, 1936); *Siteroptes graminum* (Pygmephoridae) (Cooper, 1939); *Tarsonemus randsi* (Tarsonemidae) (Beer, 1954); *Tarsinemus waitei* (= *T. setifer, T. pauperoseatus*) (Tarsonemidae) (Beer, 1954; Suski, 1972); *Pyemotes herfsi* and *P. scolyti* (Pyemotidae) (Krczal, 1959); *Iponemus confusus* and *I. radiatae* (Tarsonemidae) (Lindquist and Bedard, 1961); *Tarsonemus talpae* (Tarsonemidae) (Karl, 1965b); *Tarsonemus confusus* (Tarsonemidae) (Karl, 1965b; Suski, 1972) (one of five populations was thelytokous

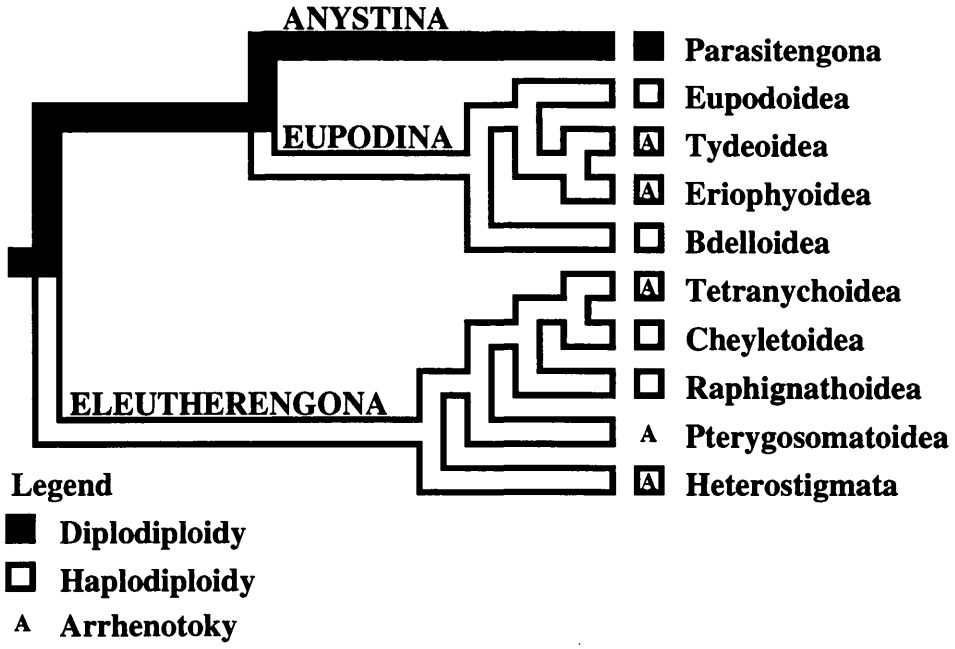

Figure 3.3: The Phylogeny of the Known Genetic Systems of The Prostigmata
Phylogenetic hypotheses are from Norton *et al.* (1993), figure 1.4, p.24.

in the Prostigmata this means that there must have been at least two independent origins of haplodiploidy in this group[55] (figure 3.3).

Although pseudoarrhenotoky has never been proven in the Prostigmata there is evidence to suggest that it could occur in the family Cunaxidae (Bdelloidea) which has haploid males and appears to have obligate mating[56]. Since the Cunaxidae are at the base of the Eupodina if they are indeed pseudoarrhenotokous then this would add a further example in support of the hypothesis of Schrader and Hughes-Schrader .

(Karl, 1965b)); *Pediculaster mesembrinae* (Pygmephoridae) (Wicht and Snetsinger, 1971); *Tarsonemus lobosus*, *T. nudosus* and *T. schlechtendali* (Tarsonemidae) (Suski, 1972); *Chrysomelobia labidomerae* (Podapolipidae) (Baker and Eickwort, 1975); *Imparipes histricinus* (Scutacaridae) (Ebermann, 1982); *Phytonemus pallidus* (Tarsonemidae) (Helle *et al.*, 1984) (some populations are arrhenotokous (Karl, 1965a) whilst others are thelytokous (Garman, 1917)); *Polyphagotarsonemus latus* (Tarsonemidae) (Flechtmann and Flechtmann, 1984); *Pediculaster flechtmanni* (Pygmephoridae) (Cross and Kaliszewski, 1988) and *Siteroptes reniformis* (Pygmephoridae) (Kaliszewski in Norton *et al.* (1993))).

[55] The most parsimonious explanation for the observed distribution of genetic systems in the Prostigmata is that arrhenotoky is plesiotypic and there has been a single reversal to diplodiploidy in the Anystina (Figure 3a). Norton *et al.* (1993), however, maintain that diplodiploidy is the plesiotypic genetic system of the Prostigmata. If this is true then there must have been at least two independent origins of arrhenotoky within the Prostigmata, one in the Eupodina and another in the Eleutherengona (Figure 3b). If the assumption made below, that plesiotypic genetic system of the Sarcoptifomes *sensu stricto* is diplodiploidy, is indeed correct then the double origin of haplodiploidy in the Prostigmata is in fact no less parsimonious than a single origin since this would require haplodiploidy to be the plesiotypic genetic system in the Prostigmata and hence imply another character state change at some point between the base of the Prostigmata and the base of the Sarcoptiformes *sensu stricto* (Figure 3) i.e. this is an optimisation problem (Swofford and Maddison, 1987)

[56] Walter and Kaplan (1991) held isolated females of *Coleoscirus simplex* in cultures for 79 days without observing oviposition suggesting that mating is obligatory. Similar results were obtained with *Neoscirula* sp. nr. *sevidi* (Norton *et al.*, 1993)).

Sarcoptiformes *sensu stricto*

Relationships amongst the taxa comprising the Sarcoptiformes *sensu stricto* is a complex and unresolved issue[57], however, two monophyletic groups can be identified which contain both diplodiploid and haplodiploid members. These are the Brachypylina and the Astigmata.[58]

Brachypylina

Of the eight families of Brachypylina (or "higher" oribatid mites) for which there are karyotype data five contain a single record of diplodiploidy[59], two, a single record of haplodiploidy[60] and one[61], a single record of each of diplodiploidy[62] and haplodiploidy[63]. In none of the cases of haplodiploidy has the exact nature of the genetic system been deduced. All three observations of haplodiploidy in this group come from a single study (Helle *et al.*, 1984). Unfortunately the methodological details in this report are insufficiently described to assess the validity of these observations. It seems that male haploidy was inferred from an approximately 1:1 ratio of haploid and diploid eggs. No mention is made of any attempt to ascertain whether haploid eggs give rise to male offspring and diploid eggs to female offspring or, indeed, whether haploid eggs are even

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[57] The Sarcoptiformes *sensu stricto* comprises two artificial groups; the Oribatida and the Astigmata. Traditionally these two groups have been considered to be of equal rank and were commonly depicted as sister taxa (e.g. Krantz (1978)), however, whilst the Astigmata is probably monophyletic it now seems likely that the Oribatida is paraphyletic with the Astigmata arising from within it (OConnor, 1984) (For this reason Norton *et al.* (1993) suggest the use of the term "oribatid mites" rather than Oribatida to represent this paraphyletic assemblage.) The oribatid mites comprise about 150 families divided into six major taxa (Grandjean, 1969); Paleostomata, Enarthronota, Parahyposomata, Brachypylina (= Circumdehiscentiae, or "higher" oribatid mites) all of which are considered to be monophyletic and the Mixonomata and Desmonomata (= Nothroidea *sensu lato*) which Norton *et al.* (1993) consider likely to be paraphyletic. The origin of the Astigmata is still very much an open question. Griffiths *et al.* (1990) Norton and Palmer (1991) and Norton (1994) have suggested that the closest living relatives of the Astigmata belong to the Desmonomata superfamily Trhypochthonoidea (an exclusively thelytokous group). They suggest that the Astigmata are a consistently over-ranked group consisting of a sexual offshoot of an ancient asexual clade (Trhypochthonoidea + Camisiidae) which has radiated into a wide range of niches in response to its freedom from the constraint of asexuality (OConnor, 1982), however, this is far from a resolved issue.

[58] Of the seven major taxa of Sarcoptiformes *sensu stricto*, male haploidy and arrhenotoky have been found in only the Brachypylina and the Astigmata; both groups also have diplodiploid members. Since these groups are both assumed to be monophyletic they can be considered separately. Of the remaining groups diplodiploidy has been identified in one species in the Enarthronota (*Hypochthonius rufulus* (2n = 18) (Hypochthoniidae) (Sokolov, 1954)) and three species in three different families of the Desmonomata (*Trhypochthonius tectorum* (2n = 18) (Tryhpochthonidae) (Taberly, 1960; Taberly, 1988) , *Platynothrus peltifer* (2n = 18; this species is thelytokous but spanandric males are diploid with normal meiosis) (Camisiidae) (Taberly, 1958a; Taberly, 1988) and *Hermannia gibba* (2n = 16) (Hermanniidae) (Taberly, 1958b). No other genetic systems have been identified in these two groups which suggests that diplodiploidy is the plesiotypic state in the Brachypylina and Astigmata, however, the relative lack of karyotype and genetic system data outside these four groups requires this hypothesis to be tentative.

[59] Damaeidae (*Damaeus verticillipes*), Achipteriidae (*Achipteria punctata*) and Euzetidae (*Euzetes globulus* (= *seminulum*)) (Sokolov, 1954) and Liododae (*Poroliodes farinosus*) and Xenillidae (*Xenillus tegeocranus*) (Taberly, 1958b) all with 2n = 18.

[60] Oppiidae (*Oppia* sp. (n = 9)) and Ceratozetidae (*Humerobates rostrolamellatus* (n = 8)) (Helle *et al.*, 1984).

[61] Galumnidae

[62] *Galumna* sp. (2n = 18) (Sokolov, 1954).

[63] *Orthogalumna terebrantis* (n = 9) (Helle *et al.*, 1984).

viable. In the absence of this information it remains possible that haploid eggs are merely inviable unfertilised eggs and both males and females arise from diploid fertilised eggs.

If, however, we were to accept the observations of haplodiploidy in the Brachypylina at face value this would suggest an extraordinary evolutionary plasticity. There is however, another possibility. Since reports of diplodiploidy have come from studies of spermatogenesis whereas reports of haplodiploidy have come from egg squashes in different but closely related species it seems more likely these reports in fact represent different temporal samples of the same genetic system. In no case have both studies of spermatogenesis and karyotyping of egg squashes been performed on the same species of oribatid mite.

The Phylogeny of the Brachypylina is poorly understood, however, some groups are well supported. The families for which genetic systems have been reported can be divided into two groups, the monophyletic Macrosclerae and the artificial group the 'nonmacrosclerous Brachypylina' (Norton *et al.*, 1993). The families Euzetidae, Ceratozetidae and Galumnidae fall into the former whilst the remainder belong to the latter. Within the Macrosclerae the Euzetidae and Ceratozetidae are probably more closely related to each other than either is to the Galumnidae. The relationships within the non-macrosclerous Brachypylina cannot, at present, be resolved. These relationships are summarised in figure 3.4.

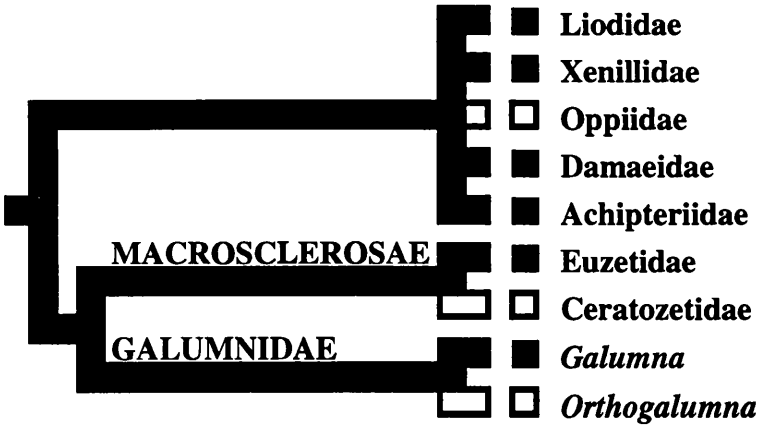

Figure 3.4: The Phylogeny of the Known Genetic Systems of the Brachypylina

When karyotypes are mapped onto these relationships the most parsimonious solution is plesiotypic diplodiploidy with three independent origins of haplodiploidy within the Brachypylina; two in the Macrosclerae (in the family Ceratozetidae and the genus *Galumna* (Galumnidae)) and one in the non-macrosclerous Brachypylina (in the family Oppiidae). With regard to haplodiploidy in *Oppia* (Oppiidae), two other species in this genus were investigated by Helle *et al.* (1984) in addition to the undetermined haplodiploid species; these were *O. concolor* and *O. bayoumi.* In both cases only diploid eggs were found but since the sex ratio of the source population was not given Norton *et*

al. (1993) point out that this may be an example of thelytoky rather than diplodiploidy. Other populations of *O. concolor* are known to be sexual (Nannelli, 1975) suggesting diplodiploidy but thelytoky does occur in the congener *O. nitens* (Sengbusch and Sengbusch (1970), Behan-Pelletier cited as pers. comm. 1990 in Norton *et al.* (1993)) suggesting the possibility that Helle *et al.* (1984) had sampled thelytokous populations of *O. concolor* and *O. bayoumi.* Apart from an erroneous report of arrhenotoky in the Oppiidae (Oliver, 1983) arrhenotoky has never been proven to be the genetic system of any oribatid mite. The observation of Woodring and Cook (1962) that virgin females of *Ceratozetoides cisalpinus* (Ceratozetidae) died of "old age" without laying eggs together with the lack of evidence for arrhenotoky in oribatid mites has led Norton *et al.* (1993) to speculate that haplodiploidy in the taxa studied by Helle *et al.* (1984) may be due to pseudoarrhenotoky. Since reports of diplodiploidy have come from studies of spermatogenesis whereas reports of haplodiploidy have come from egg squashes in different but closely related species it seems more likely that rather than these reports representing different genetic systems in a highly evolutionarily plastic group, they in fact represent temporally different samples of the same genetic system. In pseudoarrhenotoky, for example, spermatogenesis would be indistinguishable from that of a diplodiploid species but if elimination of the genome of the father occurred prior to the point at which eggs were sampled then egg squashes would reveal males to be haploid and females to be diploid. Indeed, since the parental origin of the haploid genome in males has not been elucidated this may even represent another somatic postfertilisation uniparental genetic system such as *maternal* genome loss.

Astigmata

The Astigmata consists of about 70 families in ten superfamilies[64]. Diplodiploidy has been identified in the Glycyphagoidea[65] and Acaroidea[66], haplodiploidy in the Histiostomatoidea[67] and Analgoidea[68] and arrhenotoky in the Histiostomatoidea[69] and Hemisarcoptoidea[70]. If this information is mapped onto the putative phylogenetic relationships among these taxa, taking diplodiploidy to be plesiotypic then there are two different optimisation solutions (figure 3.5).

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[64] Schizoglyphoidea, Histiostomatoidea, Canestrinioidea, Hemisarcoptoidea, Glycyphagoidea, Acaroidea, Hypoderatoidea, Pterolichoidea, Analgoidea and Sarcoptoidea.

[65] In one species; *Glycyphagus domesticus* (2n = 18) (Gycyphagidae) (Sokolov, 1945).

[66] In nine species (all in the family Acaridae) (*Acarus siro* (= *Tyroglyphus farinae*) (2n = 18) (Sokolov, 1945); *Rhizoglyphus echinopus* (2n = 10) (Grondziel, 1975; Sokolov, 1945); *Sancassania berlesi* (2n = 18) and *S. michaeli* (2n = 16) (Prasse, 1968); *S. mycophaga* (2n = 16) (Heinemann and Hughes, 1970); *Tyrophagus neiswanderi* (2n = 12) and *T. palmarum* (2n = 16) (Grondziel, 1976); *T. putrescentiae* (= *T. noxius*) (2n = 16) (Grondziel, 1976; Sokolov, 1945) and *T. casei* (2n = 10) (Zalewska and Rajski, 1981))

[67] In two species of this monofamilial superfamily (Histiostomatidae (= Anoetidae)), *Histiostoma feroniarum* (= *rostroserrata*) (n = 7)(Jary and Stapely, 1936) and *Histiostoma laboratorium* (n = 4) (Hughes, 1950))

[68] *Myialges pari* (n = 8) (Epidermoptidae) (Helle and Wysoki, 1983).

[69] In seven species; *Hististoma julorum* and *H. laboratorum* (Hughes and Jackson, 1958); *H. murchei* (Oliver, 1962); *H. feroniarum* (= *rostroserrata*)(Heinemann and Hughes, 1969); *H. formosana* (Philippsen and Coppel, 1977); *Hexanoetus conoidalis* and *Myianoetus* sp. (OConnor in Norton *et al.* (1993))

[70] In one species parasitic on wasps (*Kennethiella trisetosa* (Winterschmidtiidae (= Saproglyphidae)) based on the fact that eggs which produce small males are laid prior to mating (Cowan, 1984). This also appears to be true in two other

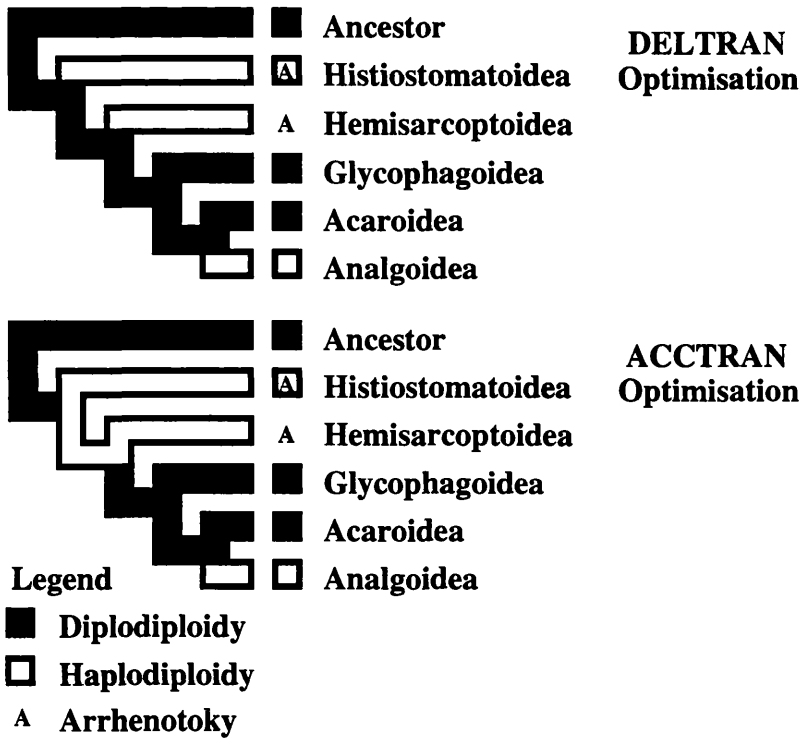

Figure 3.5: Two optimisation solutions for the phylogeny of the genetic systems of the Astigmata

Phylogenetic hypotheses are from Norton *et al.* (1993), figure 1.6, p.32. DELTRAN (delayed transformation) optimisation maximises the proportion of the homoplasy that is accounted for by parallelism and minimises that accounted for by reversals by postponing character state changes as far as possible from the root of the tree (Kitching, 1992b; Swofford and Maddison, 1987). In this case there are three parallelisms (tree length = 3). ACCTRAN (accelerated transformation) optimisation minimises the proportion of the homoplasy that is accounted for by parallelisms and maximises that accounted for by reversals by placing the character state changes on the tree as close to the root as possible (Farris, 1970). In this case there are two parallelisms and one reversal (tree length = 3).

Although pseudoarrhenotoky has never been recorded in the Astigmata there is some evidence for obligate mating in at least one species of Histiostomatoid mite congeneric with five species of arrhenotokes with sex ratios characteristic of haplodiploid genetic systems, suggesting that pseudoarrhenotoky may be found in addition to arrhenotoky in this group, however, this remains to be proven.[71]

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enslinielline winterschmidtiid genera; *Ensliniella* and *Vespacarus* (Klompen *et al.* (1987); OConnor in Norton *et al.* (1993))

[71] Hughes and Jackson (1958) noted that virgin females of *Histiostoma jacksonae* (which they apparently misidentified as *H. fimetarium* (Mahunka, 1967)) a congener of five species of arrhenotokes (see above) with sex ratios characteristic of haplodiploid genetic systems, failed to produce eggs and concluded from this that "pseudofertilisation or at least insemination is necessary to initiate development of male producing eggs." This observation led initially to an assumption of pseudogamic arrhenotoky (Oliver, 1971) and subsequently pseudoarrhenotoky (Oliver, 1983) in this species but although listed as an instance of pseudoarrhenotoky in Table 1 of Oliver (1983) it remains to be proven.

Parasitiformes

The Parasitiformes comprise the Opilioacarida, Ixodida (ticks), Holothyrida, and Mesostigmata (see figure 3.1 above). The only one of these groups in which haplodiploidy has been identified is the Mesostigmata.

Mesostigmata

The Mesostigmata comprises 11 cohorts. Karyotypes have been recorded for four of these. Diplodiploidy has been reported within the Cercomegistina[72] and Parasitina[73] whilst Haplodiploidy has been reported in the Antennophorina[74] and Dermanyssina[75] (figure 3.6). Whilst the precise genetic system underlying haplodiploidy in the Antennophorina is unknown, both arrhenotoky and pseudoarrhenotoky have been reported from a number of species within the Dermanyssina. For this reason the Dermanyssina is an appropriate group in which to test the hypothesis of Schrader and Hughes-Schrader that pseudoarrhenotoky is an intermediate step in the evolution of arrhenotoky from diplodiploidy.

Dermanyssina

The Dermanyssina has traditionally been divided into five superfamilies; Veigaioidea, Rhodacaroidea, Eviphidoidea, Dermanyssoidea and Ascoidea. Nothing is known about the genetic systems of the Veigaioidea or Rhodacaroidea. The Eviphidoidea and Dermanyssoidea contain only arrhenotokes whereas the Ascoidea contains both arrhenotokes and pseudoarrhenotokes. The phylogeny of these superfamilies is unknown

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[72] *Cercoleipus coelonotus* (Cercomegistidae) (2n = 26) (Kinn, 1971).

[73] *Pergamasus brevicornis* (2n = 12), *Amblygamasus septentrionalis* (2n = 12), *Eugamasus magnus* (2n = 10) and *E. kraepelini* (2n = 12) (Parasitidae)(Sokolov, 1934).

[74] *Antennophorus grandis* (n = 13-15/2n = 22-26) (Franks, Healey and Byrom, 1991).

[75] *Macrocheles boudreauxi* (n = 5) (Hse in Kinn and Witcosky (1977)), *Areolaspis bifoliatus* (n = 5), *Macrocheles muscadomesticae* (n = 5), *M. pisentii* (n = 5) *M. vernalis* (n = 5) (Oliver, 1977) (Eviphidoidea: Macrochelidae); *Blattisocius patagiorum* (n = 3 or 4) (Treat, 1966) (although Treat did not assume haplodiploidy in this species, Norton *et al.* (1993) cite his paper as evidence that this is the case since his chromosome counts appear to fall into two categories, 3-4 and 6-8), *Lasioseius subterraneus*, *Proctolaelaps krimesi*, *P. longipilis* and *Rhinoseius colwelli* (determined by electrophoresis rather than karyotype) (Kaliszewski cited as unpublished in Norton *et al.* (1993)) (Ascoidea: Ascidae); *Dicrocheles phalaenodectes* (n = 3) (Treat, 1965) (Ascoidea: Otopheidomenidae); *Typhlodromus* (formerly *Clavidromus*) aff. *jackmickleyi* (Amitai, Wysoki and Swirski, 1969; Oliver, 1977), *Amblyseius judaicus* (n=4), *A. messor* (n=4) (Amitai and Wysoki, 1974; Oliver, 1977), *A. bibens* (n=4), *A. brevipes* (n=4), *A. masiaka* (n=4), *A. rotundus* (n=4), *A. vazimba* (n=4), *Phytoseius amba* (n = 4), *Typhlodromus chazeaui* (n = 4), *T. gutierrezi* (n = 4) (Blommers-Schlosser and Blommers, 1975; Oliver, 1977), *Typhlodromus caudiglans* (n = 4), *T. fallacis* (n = 4) (Hansell, Mollison and Putman, 1964; Oliver, 1977), *Phytoseiulus persimilis* (n = 4) (Hansell *et al.*, 1964; Oliver, 1977; Wysoki, 1973; Wysoki and Swirski, 1968), *Amblyseius cucumeris* (n=4) (Oliver, 1977; Treat, 1965; Wysoki, 1973), *A. aberrans* (n=4), *A. barkeri* (n=4), *A. chiapensis* (n=4), *A. deleoni* (n=4), *Paragignathus tamaricis* (n = 4?), *Phytoseius finitimus* (n=4), *Typhlodromus contiguus* (n=4), *T. drori* (n=4), *T. phialatus* (n=4), *T. porathi* (n=4), *T. sternlichti* (n=4), *Typhloseiulus* (formerly *Seiulus*) *isotrichus* (n=4) (Oliver, 1977; Wysoki, 1973), *Amblyseius chilensis* (n=4), *A. hibisci* (n=4), *A. largoensis* (n=4), *A. rubini* (n=4), *A. swirskii* (n=4), *Iphiseius degenerans* (n = 4), *Typhlodromus athiasae* (n = 4), *T. occidentalis* (n = 3), *T. rhenanus* (n = 4) (Oliver, 1977; Wysoki and Swirski, 1968) (Ascoidea: Phytoseiidae); *Podocinum sagax* (n = 5) (Oliver, 1977; Wong, 1967) (Ascoidea: Podocinidae); *Eulaelaps shanghaiensis* (n = 8) (Chen and Meng, 1988) (Dermanyssoidea: Haemogamasidae); *Androlaelaps casalis* (n = 7) (Chen and Meng, 1987), *Geolaelaps aculeifer* (n = 9), *Stratiolaelaps miles* (n = 7) (DeJong *et al.*, 1981), *Hypoaspis lubrica* (n = 7) (Li and Meng, 1990), *Varroa jacobsoni* (n = 7) (Steiner *et al.*, 1982), *Cosmolaelaps gurabensis* (n = 6) (Zhou and Meng, 1982) (Dermanyssoidea: Laelapidae); *Dermanyssus gallinae* (n = 3), *D. Prognephilus* (n = 3) (Oliver, 1977) (Dermanyssoidea: Dermanyssidae); *Ornithonyssus bacoti* (n = 8) (Oliver, 1965; Zhou and Meng, 1982), *O. silviarum* (n = 9) (Oliver, 1965), *Ophionyssus natracis* (n = 9) (Oliver, Camin and Jackson, 1963) (Dermanyssoidea: Macronyssidae).

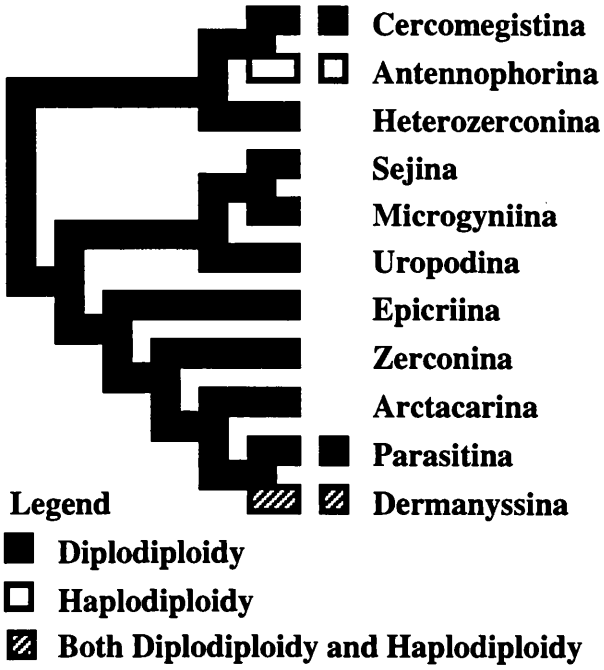

Figure 3.6: The Phylogeny of the known genetic systems of the Mesostigmata
Phylogenetic hypotheses are from Norton *et al.* (1993), figure 1.2, p.15.

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and therefore represents a good test of the hypothesis of Schrader and Hughes-Schrader which would predict that the arrhenotokous members of the Ascoidea together with the Eviphidoidea and Dermanyssoidea form a single monophyletic clade which arose from a pseudoarrhenotokous ancestor at the base of the Ascoidea.

The Plesiotypic State

Male haploidy in the Parasitiformes is restricted to the Mesostigmata. The most plausible outgroup to the Mesostigmata is the Ixodida (= Metastigmata) (the ticks). All ticks in which the genetic system is known are diplodiploid with obligate mating (Norton *et al.*, 1993; Oliver, 1977). Females are always XX and males are either XY or XO. This is the plesiotypic state in the Mesostigmata from which male haploidy is likely to have arisen a number of times. Male haploidy may be more likely to evolve from systems in which males are XO than ones in which males are XY since in XO systems any viable haploids which arise in the population would be male due to hemizygosity at the sex determining locus whereas in XY systems any viable haploids would be female since YO is likely to be inviable and XO would be female due to lack of a male specifying locus on a Y chromosome.

Arrhenotoky

Within the Dermanyssina, arrhenotoky occurs in the Eviphidoidea, Dermanyssina and Ascoidea. Diplodiploidy is unknown in these superfamilies but the Ascoidea contains pseudoarrhenotokes as well as arrhenotokes.

Eviphidoidea

Arrhenotoky has been demonstrated in at least 23 species in the Eviphidoid family Macrochelidae (in four out of five genera)[76] by the rearing of male only progeny from virgin females and arrhenotoky has been considered likely to be the genetic system of all phoretic macrochelids. Krantz and Royce (1994) however have recently shown an interesting exception to arrhenotoky in the phoretic macrochelid *Macrocheles mycotrupetes*. This species has a morphology more typical of primitive free living macrochelids than of other phoretic forms and it seems likely that the phoretic association of *Macrocheles mycotrupetes* with the scarab beetle *Mycotrupetes gaigei* has evolved independently of other macrochelid/scarab phoretic associations. *Macrocheles mycotrupetes* does not produce any progeny in the absence of mating, and, unlike other phoretic macrochelids, produces a sex ratio of 1:1. The species has not yet been karyotyped but these results suggest a diplodiploid or parahaploid type of genetic system rather than the usual arrhenotokous genetic system of the phoretic macrochelids. Norton *et al.* (1993) suggested that the evolution of phoresy is often a prelude to the evolution of haplodiploidy. *Macrocheles mycotrupetes* may therefore represent a separate phoretic lineage derived from and still possessing the diplodiploid genetic system of the free living ancestor of the rest of the phoretic macrochelids. The evolution of phoresy in this primitive lineage has not been accompanied by the evolution of arrhenotoky, possibly due to the restricted gallery habitat or other life history parameters of its major beetle phoront *Mycotrupetes gaigei*. The genetic systems of other families within the Eviphidoidea are unknown.

Dermanyssoidea

Arrhenotoky has been reported in two families within the Dermanyssoidea, Laelapidae[77] and Macronyssidae[78]. Diplodiploidy and pseudoarrhenotoky are unknown in this superfamily.

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[76] *Areolaspis bifoliatus* (Oliver, 1971), *Glyptholaspis fimicola* (Filipponi, 1955), *G. confusa* (Filipponi, 1958), *G. americana*, *G. pontina*, *Holostaspella* sp., *Macrocheles mammifer*, *M. matrius*, *M. merdarius*, *M. scutatus* (Filipponi, 1964), *M. parapisentii* (Costa, 1967), *M. robustulus* (Costa, 1966; Filipponi, 1964)), *M. subbadius* (Filipponi, 1964; Filipponi and Seganti, 1957), *M. pisentii* (Filipponi, 1964; Oliver, 1971), *M. vernalis* (Cicolani, 1980; Filipponi, 1964; Oliver, 1971), *M. perglaber* (Filipponi, 1964; Halliday and Holm, 1985), *M. glaber* (Filipponi and Cervone, 1957), *M. insignitus* (Filipponi, 1964; Filipponi and Ilardi, 1959), *M. peregrinus* (Halliday and Holm, 1985), *M. boudreauxi* (Hse in Kinn and Witcosky (1977)), *M. rodriguezi* (Oliver and Krantz, 1963), *M. muscadomesticae* (Filipponi, 1964; Oliver and Krantz, 1963; Pereira and Castro, 1947; Wade and Rodriguez, 1961), *M. schaeferi* (Walter, 1988).

[77] *Haemogamasus centrocarpus*, *H. longipes* (Furman, 1966), *Geolaelaps aculeifer* (Usher and Davis, 1983; Walter and Oliver, 1989), *Cosmolaelaps* sp. nr. *weeversi*, two unknown species of *Geolaelaps*, *Stratiolaelaps miles* (Walter and Kaplan, 1990), *Cosmolaelaps* n. sp., *Euandrolaelaps* sp. nr. *karawaiewi*, *Laelaspis* sp. nr. *vitzthumi*, *Pseudoparasitus* sp. (Walter cited as unpublished in Norton *et al.* (1993)).

[78] *Ornithonyssus bacoti* Ohmori (1936), Bertram *et al.* (1946), Skaliy and Hayes (1949), Nelzina in Evans *et al.* (1961) and Oliver (1965), *O. silviarum* (Oliver, 1965), *Ophionyssus natracis* (Camin, 1953; Oliver *et al.*, 1963).

Ascoidea

The Ascoidea is the only superfamily of the Dermanyssina known to contain pseudoarrhenotokes, however it also contains arrhenotokes[79]. These are restricted to the genus *Gamasellodes* within the family Ascidae. Unfortunately the genetic systems of other genera within this family are unknown but if the hypothesis of Schrader and Hughes-Schrader is correct then at least the genus *Gamasellodes*, if not the whole of the Ascidae, should form a monophyletic clade with the Eviphidoidea and Dermanyssina. If the large superfamilies Eviphidoidea and Dermanyssoidea did indeed arise from within the genus *Gamasellodes* then this genus is of considerable interest since the switch in genetic system associated with its formation may have been the evolutionary innovation which allowed the radiation of these two superfamilies.

Pseudoarrhenotoky

Although pseudoarrhenotoky has been reported in both Acariform and Parasitiform mites it has only been confirmed within the Parasitiformes. Treat (1965) has demonstrated pseudoarrhenotoky in the moth ear mite *Dicrocheles phalaenodectes* (Ascoidea: Otopheidomenidae). It was known that virgin females of this haplodiploid mite laid only inviable eggs. Since this species has haploid males this observation had previously been given as evidence for pseudogamous arrhenotoky. Treat, however, found that the haploid viable eggs of mated females contained heterochromatic masses which were never found in diploid eggs and were retained by males but not by females. Eggs containing heterochromatic masses were found in the tympanic air sac of the host moth where the first males of a colony are known to develop. These observations suggested that the heterochromatic masses were the remains of the paternal genome and that males arise by pseudoarrhenotoky rather than pseudogamous arrhenotoky. In the same paper Treat also reported the first evidence for pseudoarrhenotoky in the family Phytoseiidae (Ascoidea) (also previously thought to exhibit pseudogamous arrhenotoky) by the identification of heterochromatic bodies in embryonic cells of haploid males of the species *Amblyseius cucumeris*. Further evidence for pseudoarrhenotoky in the Phytoseiidae was given by Helle *et al.* (1978) (in *Amblyseius bibens* and *Phytoseiulus persimilis*) and Hoy (1979) (in *Typhlodromus occidentalis*) who demonstrated that the genotype of the father could affect the genotype of the son by showing the presence of radiation damage in male progeny of irradiated haploid fathers. Pseudoarrhenotoky is now considered likely to be the genetic system of this entire family.

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[79] *Gamasellodes rectiventris* (Walter and Kaplan, 1990), *G. vermivorax* (Walter and Ikonen, 1989; Walter, Kethley and Moore, 1987), *Gamasellodes* n. sp. (Walter and Ikonen, 1989).

Chapter Four
Materials and Methods

INTRODUCTION

DNA was extracted and the region to be sequenced was amplified using the polymerase chain reaction (PCR). The primary amplification product was run on an agarose gel to check for purity and yield. If the yield was low the product was reamplified to produce a secondary amplification product for sequencing, otherwise the primary amplification product was used. If the purity was low then the product was gel purified prior to sequencing. The amplification product was then cleaned up and the DNA concentration measured. This product was then cycle sequenced directly by either manual or automated methods.

SOURCES OF MITES

The sources of all of the mites used in this project are shown in table 4.1.

DNA EXTRACTION

Seven different methods of extracting DNA were used. Methods 1, 2 and 4 below are modifications of a method developed by Neil Shailer at the Natural History Museum, London whilst methods 3, 5, 6 and 7 are derived from a method developed by Ilik Sacheri of the Department of Genetics at the Institute of Zoology, Regents Park and modified by Debbie Goode, also of the Natural History Museum. Table 4.1 lists the extraction method used for each individual sequenced[80]. Protocols for these seven extraction methods are given in appendix 4.1. Of the fourteen species sampled two (one ingroup taxon (*Stratiolaelaps miles*) and one outgroup taxon (*Pergamasus septentrionalis*) were done in duplicate to check for intraspecific polymorphisms. In both cases the entire sequences were identical.

PRIMARY AMPLIFICATION

Primary amplification was performed according to the protocol given in appendix 4.2. The primers used for primary amplification were taken from Friedrich and Tautz (1995) and are listed in table 4.3.

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[80] Other methods for DNA extraction which were attempted but ultimately rejected included the methods of Kaliszewski *et al.* (1992), Rose *et al.* (1994), and Phillips and Simon (1995).

Table 4.1: Sources of Mites[81]

Species	Collector*	Institution
Amblyseius cucumeris	Dr. A. Baker	Natural History Museum
Cornigamasus lunaris	Dr. A. Baker	Natural History Museum
Veigaia nemorensis	Dr. A. Baker	Natural History Museum
Stratiolaelaps miles	Dr. C. S. A. Stinton	Bunting Biological Control[82]
Pergamasus septentrionalis	Mr. R. H. Cruickshank	Natural History Museum
Macrocheles glaber	Mr. R. H. Cruickshank	Natural History Museum
Dermanyssus gallinae	Dr P. D. Hillyard	Natural History Museum
Phytoseiulus persimilis	Dr. A. Baker	Natural History Museum
Spinturnix myoti	Dr. G. Jones**	University of Bristol[83]
Spinturnix plecotinus	Dr. E. Barratt**	Institute of Zoology
Typhlodromus pyri	Dr. J. Fitzgerald	HRI[84]
Hypoaspis rosei	Dr. K. L. Strong	CSIRO[85]
Varroa jacobsoni	Dr. P. Wilkins	National Bee Unit[86]
Hemipteroseius wormersleyi	Dr. Z. Zhang	Institute of Entomology[87]

* Unless stated otherwise the species identification was determined by the collector.

** Species identification determined by Mr. R. H. Cruickshank.

AGAROSE GEL ELECTROPHORESIS OF PRIMARY AMPLIFICATION PRODUCT

Agarose gel electrophoresis of the primary amplification product was performed according to the protocol given in appendix 4.3.

SECONDARY AMPLIFICATION (where necessary)

For mites which did not yield enough DNA on primary amplification for all of the necessary sequencing, a secondary amplification was performed. The products of the primary amplification were run out on a low melting point agarose gel. A pipette tip was stabbed into the area of the gel containing the band corresponding to the primary

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[81] Mites were also gratefully received from Dr. A. Pacejka, Ecology Group, Department of Biology, Illinois State University, Normal, IL 61761-6901, USA (*Dermanyssus hirundinis*, *Androlaelaps casalis*), Dr. P. W. Richardson, Bat Conservation Trust, 10 Bedford Cottages, Great Brington, NORTHAMPTON NN7 4JE (*Spinturnix myoti*), Dr. O. Seeman, Australia (*Hattena cometis*), J. A. McKenzie-Dodds, Natural History Museum (*Hirstionyssus* sp.) but these were not used in the final analysis.

[82] Bunting Biological Control Ltd., Westwood Park, Little Horkesley, COLCHESTER, Essex CO6 4BS, England, UK.

[83] University of Bristol, School of Biological Sciences, Woodland Road, BRISTOL BS8 1UG, England, UK.

[84] Horticulture Research International, East Malling, WEST MALLING, Kent ME19 6BJ, England, UK.

[85] CSIRO Division of Entomology, GPO Box 1700, Canberra, ACT 2601, Australia.

[86] National Bee Unit, Central Science Laboratory, Ministry of Agriculture, Fisheries and Food, Luddington, STRATFORD-UPON-AVON, Warwickshire CV37 9SJ, England, UK.

[87] International Institute of Entomology, 56 Queen's Gate, South Kensington, LONDON SW7 5JR, England, UK.

Table 4.2: DNA Extraction Method Used for Each Individual in the 28S rDNA Data Set

Species	Individual	Method
Amblyseius cucumeris	1	1
Cornigamasus lunaris	1	1
Veigaia nemorensis	1	2
Stratiolaelaps miles	1	3
Stratiolaelaps miles	2	3
Pergamasus septentrionalis	1	4
Pergamasus septentrionalis	2	5
Macrocheles glaber	1	6
Dermanyssus gallinae	1	6
Phytoseiulus persimilis	1	6
Spinturnix myoti	1	6
Spinturnix plecotinus	1	6
Typhlodromus pyri	1	6
Hypoaspis rosei	1	6
Varroa jacobsoni	1	7
Hemipteroseius wormersleyi	1	7

Table 4.3: Primers used for Primary Amplification

Primer	Direction	Sequence	t_{anneal}
rD3A	Forward	CCCGAAAGATGGTGAACTAT	58°C*
rD7B1	Reverse	GACTTCCCTTACCTACAT	52°C*

*Annealing temperatures were estimated using Sugg's rule, t_{anneal} = (4 x (the number of Cs in the primer + the number of Gs in the primer)) + (2 x (the number of As in the primer + the number of Ts in the primer)). A compromise annealing temperature of 55°C was used initially but this was later dropped to 45°C when it was found that this increased the yield without apparently decreasing the specificity.

amplification product and this tip was then agitated in a tube containing all the same components as in the primary amplification except the template DNA. The same thermal cycle was used as in the primary amplification.

GEL PURIFICATION (where necessary)

Gel purification was performed on amplification products which appeared to contain extra bands or smearing to remove DNA with molecular weights other than those of the expected amplification product. This was performed using the QIAEX II kit (from QIAGEN) with some modifications from the manufacturers protocol. This modified protocol is given in appendix 4.4.

CLEANING UP AND CONCENTRATING THE PCR PRODUCT FOR SEQUENCING

Amplification products were cleaned up using a chloroform extraction and concentrated using a Microcon-100 microconcentrator (from Amicon) with some modifications from the manufacturers protocol. The protocols used are given in appendix 4.5

MEASUREMENT OF DNA CONCENTRATION

Prior to sequencing the concentration of DNA was measured using a spectrophotometer. The absorbence (A) was measured twice for each sample at each of two wavelengths, 280 nm and 260 nm. A280 measures the amount of protein in the sample whilst A260 measures the amount of nucleic acid. For double stranded DNA the amount of DNA in ng/µl the sample = mean A260 x 50 (x the dilution factor). The purity of the sample is given by the expression mean A260 / mean A280. This value should be between 1.8 and 2.0.

DIRECT CYCLE SEQUENCING - I. MANUAL

Direct manual cycle sequencing was performed on most samples in addition to automated sequencing which was performed on all samples. Direct sequencing gives a statistical average sequence for all copies of that sequence in the sample. Since all samples came from whole single mites the sequences generated represent an average sequence for all copies of the 28S gene from a single mite. The method used was based on that of Embley (1991).

Primer Labelling

Primers were 5' end labelled with γ^{32}-P prior to sequencing using the Tested User Friendly T4 Polynucleotide Kinase protocol (from USB).

Sequencing Reactions

Sequencing reactions were based on a modification of the TAQuence Cycle-Sequencing kit (from USB (Product number 71075)) using the reagents provided with that kit. This

Table 4.4: Primers used for Sequencing

Primer	Direction	Sequence	t_{anneal}
D3-5			
rD3C	Forward	CCCGAAAGGATGGTGAACTAT	58°C
rD3B	Reverse	TCGGAAGGAACCAGCTACTA	60°C
rD5A	Reverse	CGCCAGTTCTGCTTACC	54°C
rD5B2	Reverse	ACACACTCCTTAGCGGA	52°C
D7			
rD7A2	Forward	AGGGTTTCGTGTGAACAG	54°C
rD7B1*	Reverse	GACTTCCCTTACCTACAT	52°C

* Also a primary amplification primer.

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protocol is given in appendix 4.6 .The primers used for sequencing were taken from Friedrich and Tautz (1995) and are listed in table 4.4.

ACRILAMIDE GEL ELECTROPHORESIS OF MANUAL SEQUENCING PRODUCTS

Acrilamide gel electrophoresis of the manual sequencing products was performed according to the protocol given in appendix 4.7

DIRECT CYCLE SEQUENCING - II. AUTOMATED

Although some samples were sequenced manually using ^{32}P, all were sequenced using an ABI 373 automated flourescent sequencer. Those samples for which both methods were used gave the same sequence in all cases. however, automated sequencing was able to resolve some bases which could not be resolved using manual sequencing. Cycle sequencing was performed using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit With AmpliTaq DNA polymerase, FS (from Perkin Elmer) on an MJ thermal sequencer. Extension products were cleaned up using AGTC Centriflex Gel Filtration Cartridges (from Advanced Genetics Technologies Corporation (Catalog Number 42453). Manufacturers protocols were followed exactly without modification and are therefore not reproduced here. Purified extension products were air dried and run on an ABI 373 at the Institute of Zoology by Dada Gottelli[88].

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[88] Conservation Genetics Group, Institute of Zoology, Zoological Society of London, Nuffield Building, Regent's Park, LONDON NW1 4RY.

Chapter Five

A Phylogenetic Analysis of 28S Ribosomal DNA in the Dermanyssina (Acari: Mesostigmata)

SUMMARY

In chapter 3 it was shown that the Dermanyssina (Acari: Mesostigmata) is an ideal group in which to test the hypothesis of Schrader and Hughes-Schrader. It was also shown that the necessary missing component required for such a test is a reliable hypothesis of phylogenetic relationships within this group. Since there is no consensus whatsoever on the phylogeny within the Dermanyssina, a molecular phylogeny of the group was constructed which would sample all of the relevant genetic systems.

Two regions of 28S ribosomal DNA were sequenced. After removal of unalignable regions and regions of unreadable sequence this consisted of a data set containing 755 base pairs of aligned sequence for each of 16 individuals representing 14 species (see appendix 5.1). Two species (one ingroup species and one outgroup species) were represented by two individuals in order to assess the level of intraspecific polymorphism. In both cases the two sequences were identical. A g1 statistic derived from 10000 random trees constructed from this data was highly significant indicating that the data set contains genuine hierarchical structure and that it can be used to make valid phylogenetic inferences.

Of the 755 base pairs 136 were variable and 91 were informative according to the parsimony criterion. The proportion of variable sites is low and transitions are twice as frequent as transversions which means that the sequences are not saturated (Brown *et al.*, 1982; DeSalle *et al.*, 1987; Higuchi *et al.*, 1984; Higuchi *et al.*, 1987; Holmquist, 1976; Holmquist, 1983; Li, 1997; Li and Graur, 1991) i.e. the number of superimposed substitutions (multiple hits) should be low and similarities between sequences are likely to reflect common ancestry (i.e. phylogeny) rather than random noise.

An exhaustive parsimony search produced four equally parsimonious trees. None of these trees could be removed from the set of maximally parsimonious trees by filtering to remove any polytomous trees for which a more highly resolved compatible tree exists. Nine different a posteriori weighting schemes were tried in order to decrease the size of the set of maximally parsimonious trees. Characters were reweighted by the maximum, mean and minimum values of the consistency indices (CI), retention indices (RI) and rescaled consistency indices (RC = CI x RI) calculated from the initial heuristic search. In

each case a single tree was found but it was not always the same tree. It was however always one of two trees suggesting that these two trees were more supported by the data than the other two. A strict consensus of these two trees, however, was no more resolved than a strict consensus of all four of the original trees. An Adam's consensus of these two trees was more resolved than an Adam's consensus of all four trees, however, this contained groups which were not present in any of the four original trees. For this reason a strict consensus of the four maximally parsimonious trees was considered the best summary of the phylogenetic hypotheses supported by the data. (A semi-strict consensus was no more resolved than a strict consensus for either all four maximally parsimonious trees or for the two produced by the a posteriori weighting schemes). The unresolved portion of the tree consists of a clade containing all the arrhenotokous species. This clade arises within a clade consisting of the pseudoarrhenotokous species. The position of the arrhenotokous clade is stable, it is only the relationships within the clade that are unstable. This tree therefore provides support for the hypothesis of Schrader and Hughes-Schrader.

Distance based methods (Neighbour Joining) and Maximum Likelihood analyses were also performed. In no case were these in conflict with the hypothesis of Schrader and Hughes-Schrader although they differed in degree of resolution they offered. Some provided support for the hypothesis of Schrader and Hughes-Schrader whilst others did not but in general it was the trees based on the more realistic assumptions which were the ones which gave support for this hypothesis.

INTRODUCTION

The phylogenetic analysis of a data set has four components. Firstly it is sensible to ask of the data whether it contains any phylogenetic signal at all. Data sets which are constructed by randomly assigning character states to taxa will often support some hypotheses of relationships over others, however, this is due to the stochastic nature of the sampling process rather than common ancestry. The second phase is phylogenetic reconstruction in which some subset of trees is chosen from the set of all possible trees. The principle of phylogenetic reconstruction is to identify the subset of all possible trees which are most favoured by the data. Ideally this subset will contain a single tree but it may not be possible to choose between a number of different hypotheses based on the data available. Methods for phylogenetic reconstruction can be broadly divided into two main types, those in which a tree is constructed according to the operation of some algorithm the data and those in which the tree or trees which satisfy some optimality criterion are found from the set of all possible trees. Since algorithmic methods have no explicit optimality criteria it is much more difficult to use these methods to compare different tree topologies. The third phase consists of testing the reliability of the reconstruction as an inference from the data i.e. the degree to which the data support one set of hypotheses over another. This is not a measure of how well the phylogeny

represents genuine relationships but only how decisive the data are. The data may strongly support the wrong conclusion or only weakly support the right conclusion. This phase tests the strength of that support rather than its concordance with the truth. Methods for testing reliability can be divided into two groups; those specific to a particular method of phylogenetic reconstruction (which are discussed below alongside the discussion of the method in question) and those independent of the method of reconstruction (which are discussed in a separate section). These methods include character resampling (bootstrapping[89] and jacknifing[90]), taxon resampling and comparison of the results of different reconstruction methods (with different assumptions). The fourth phase consists of testing the degree to which the phylogenetic reconstructions fit the observed world[91] (Baum and Larson, 1991; Coddington, 1988; Donoghue, 1989; Doolittle, 1994; Doyle and Donoghue, 1986; Funk and Brooks, 1996; Harvey *et al.*, 1996; Harvey and Pagel, 1991; Lemen and Freeman, 1989; Losos, 1996b; Maddison and Maddison, 1992; Mayr and Ashlock, 1991; Quicke, 1993). This phase assesses how reasonable the phylogenetic reconstruction is i.e. how many other well founded hypotheses would have to be refuted if the phylogenetic reconstruction were correct.

This is not the only approach to phylogenetic analysis, for example, Wheeler (1991) suggests that the sorts of information consulted in phase four in order to test the phylogenetic reconstruction against the real world, be consulted at stage two and used to establish prior probabilities in a Bayesian analysis.

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[89] Bootstrapping is method of resampling characters in which a new data set is derived from the original by sampling characters with replacement until the new data set contains the same number of characters as the original (Efron, 1985; Efron and Tibshirami, 1993; Felsenstein, 1985a; Hillis and Bull, 1993; Sanderson, 1995). A number of data sets are constructed in this manner and these are used to construct trees. The proportion of trees constructed which contain a particular group is the bootstrap proportion for that node corresponding to that group. Bootstrap proportions below 50 are generally rejected and the node is collapsed to an unresolved node so that a bootstrap tree is the same as a 50 % majority consensus of the resampled data sets.

[90] Jacknifing is also a character resampling methods and differs from bootstrapping only in the way that resampled data sets are constructed. In jacknifing each character in the original data set is visited and either chosen for the new data set or rejected from it with a certain probability. There is much argument in the literature over the relative benefits of bootstrap and jacknife. By choosing the probability of rejecting a character carefully it is claimed by some authors that the statistical properties of the jacknife can be made more explicit than those of the bootstrap. This literature has been reviewed by Efron (1979; 1982), Efron and Gong (1983) and Wu (1986).

[91] This could take many forms, for example comparison with the phylogenetic relationships inferred by other data sets (Baldwin *et al.*, 1991; de Queiroz, Donoghue and Kim, 1995; Huelsenbeck, Bull and Cunningham, 1996; Meyer *et al.*, 1990; Patterson, 1987; Patterson, Williams and Humphries, 1993), fossil evidence (Donoghue *et al.*, 1989; Forey, 1992; Fortey and Jeffries, 1982; Patterson, 1981), coevolutionary evidence (Bateman *et al.*, 1990; Brooks and McClennan, 1993; Martin, Somerville and Loiseux de Goer, 1992; Page and Hafner, 1996; Sogin, 1991), experimental evidence (Losos, 1996a; McLennan, 1996), biogeography (Humphries, 1992; Malhotra *et al.*, 1996; Nelson and Platnik, 1981), molecular biology (Avise, 1994; Avise, Nelson and Sugita, 1994; Hendy and Penny, 1993; Hillis, Allard and Miyamoto, 1993; Hillis and Dixon, 1991; Kreitman, 1992; Martignetti and Brosius, 1993; O'Brien and Clegg, 1993; Olsen and Woese, 1993; Penny *et al.*, 1990; Roush, 1995; Wistow, 1993), morphology (Bateman and Dimichele, 1994; Briggs, Fortey and Wills, 1992), ontogeny (Bateman, 1994; Fink, 1982; Gould, 1977; Kluge and Strauss, 1985; Mooi, 1990; Weston, 1988), ecology (Eggleton and Vane-Wright, 1994; Harvey, 1996; Wanntorp *et al.*, 1990), physiology (Emerson, 1996), life history (Smith, Littlewood and Wray, 1996; Wray, 1996), or behaviour (Basolo, 1996; Carpenter, 1989; Martins, 1996).

THE 28S RIBOSOMAL DNA DATA SET

Taxa

The 12 ingroup taxa and 2 outgroup taxa represented in the molecular phylogeny are listed in table 5.1. Sources of these mites and methods of DNA extraction are given in chapter 4 in tables 4.1 and 4.2 respectively.

Table 5.1: Taxa Included in the Phylogeny

Cohort Parasitina (outgroup)

Species	Subfamily	Family
Pergamasus septentrionalis	Pergamasinae	Parasitidae
Cornigamasus lunaris	Parasitinae	Parasitidae

Cohort Dermanyssina (ingroup)

Species	Family	Superfamily
Veigaia nemorensis	Veigaiidae	Veigaioidea
Macrocheles glaber	Macrochelidae	Eviphidoidea
Amblyseius cucumeris	Phytoseiidae	Ascoidea
Phytoseiulus persimilis	Phytoseiidae	Ascoidea
Typhlodromus pyri	Phytoseiidae	Ascoidea
Hemipteroseius wormersleyi	Otopheidomenidae	Ascoidea
Stratiolaelaps miles	Laelapidae	Dermanyssoidea
Hypoaspis rosei	Laelapidae	Dermanyssoidea
Dermanyssus gallinae	Dermanyssidae	Dermanyssoidea
Spinturnix myoti	Spinturnicidae	Dermanyssoidea
Spinturnix plecotinus	Spinturnicidae	Dermanyssoidea
Varroa jacobsoni	Varroidae	Dermanyssoidea

Cornigamasus lunaris (Parasitinae) and *Pergamasus septentrionalis* (Pergamasinae) both from the monofamilial cohort Parasitina (Parasitidae) were used as outgroups throughout. Two of the taxa listed in table 5.1 were sequenced for each of two individuals to assess the degree of intraspecific variation; these were the outgroup taxon *Pergamasus septentrionalis* and the ingroup taxon *Stratiolaelaps miles*. In both cases the sequences from the two individuals were identical. The two Spinturnicids (*Spinturnix myoti* and *S. plecotinus* (Dermanyssina: Dermanyssoidea: Spinturnicidae)) which are on the longest branch of the tree were excluded from some of the analyses but since the genetic systems of the Spinturnicidae are unknown this will not affect any test of the hypothesis of Schrader and Hughes-Schrader, or any other hypothesis of genetic system evolution. Considering the large number of species in the Dermanyssina this taxonomic sampling is

sparse, however, it does include all of the genetic systems of the group (including *Stratiolaelaps miles* which has a heterochromatised chromosome arm which has been interpreted as the vestiges of the heterochromatised chromosome complement of a pseudoarrhenotokous ancestor (DeJong *et al.*, 1981)) and therefore will provide a valid test of the hypothesis of Schrader and Hughes-Schrader.

Outgroup Taxa

Pergamasus septentrionalis

This is a large predatory mite from a compost heap in Southern England. For more information on this species see Bhattacharyya (1963). Since this mite is congeneric with a known diplodiploid[92] and no member of the cohort Parasitina has ever been shown to have any genetic system other than ancestral zygogenetic diploidploidy (see chapter 3), the genetic systems of both *Pergamasus septentrionalis* and *Cornigamasus lunaris* (see below) are assumed for the purposes of testing the hypothesis of Schrader and Hughes-Schrader to be ancestral zygogenetic diploidploidy. If this assumption proves to be unfounded then many of the consequences for genetic system evolution inferred from this phylogeny will have to be revised.

Cornigamasus lunaris

This is a large predatory mite of compost heaps, vegetable refuse, manure and haystacks[93] in most of Northern Europe (Hyatt, 1980). For reasons given above (see section on *Pergamasus septentrionalis*) this mite is assumed for the purpose of testing the hypothesis of Schrader and Hughes-Schrader to be diplodiploid.

Ingroup Taxa

Veigaia nemorensis

This is a large predatory mite of the soil, moss, litter and humus which exhibits a marked degree of phenotypic plasticity and may be found in a wide range of soil types (Evans *et al.*, 1961). The genetic system of this mite is unknown but since virgin females do not produce offspring (Evert E. Linquist pers. comm.) it is unlikely to be arrhenotokous.

Macrocheles glaber

This is a coprophilic mite often associated with coprophagous beetles[94], the nests and

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[92] *Pergamasus brevicornis* (2n = 12) (Sokolov, 1934).

[93] *C. lunaris* has been recorded from decaying seaweed (Halbert, 1915; Halbert, 1920) but there is some question about whether this is a normal habitat for this mite (see Hyatt (1980)).

[94] *M.. glaber* has been recorded from the dor beetles *Geotrupes stercorosus* (the lousy watchman), *G. mutator, G. pyrenaeus, G. spiniger, G. vernalis* and *Typhaeus typhoeus* (Geotrupidae) and the scarab beetles *Aphodius rufipes* and *A. scybalarius* (Scarabaeidae). It has also been collected from burying beetles *Nicrophorus humator* and bumble bees (*Bombus agrorum*) (Hyatt and Emberson, 1988).Adult female are phoretic on beetles and may feed on scraps of food adhering to the setae of the mouthparts of their phoronts (Evans *et al.*, 1961). Since they are arrhenotokous adult

corpses of various mammals[95] as well as manure and rotting vegetation[96] where they are predators of the eggs and young larvae of muscid flies, as well as nematodes and small enchytraeid worms (Hyatt and Emberson, 1988). It is widespread throughout Europe and the Mediterranean area (Filipponi and Pegazzano, 1962) (including England, Scotland, Wales and Ireland (Hyatt and Emberson, 1988)) and has also been recorded from Russia (Bregetova and Koroleva, 1960), USA (Axtell, 1963) and New Zealand (Emberson, 1973). The individual represented in this phylogeny came from a compost heap in southern England. *Macrocheles glaber* is arrhenotokous (Filipponi and Cervone, 1957).

Typhlodromus pyri

This pseudoarrhenotokous predatory mite is found on apple trees throughout the world and is used for the biological control of the European red mite, *Panonychus ulmi* and the apple rust mite, *Aculus schlechtendali* (McMurtry, 1982). Since pseudoarrhenotoky is assumed to be the genetic system of the entire family Phytoseiidae, *Typhlodromus pyri* is assumed for the purposes of testing the hypothesis of Schrader and Hughes-Schrader to be pseudoarrhenotokous.

Phytoseiulus persimilis

This predatory mite is by far the most widely used agent of biological control of mites in greenhouses (McMurtry, 1982), an enterprise which represents considerable economic interests. *Phytoseiulus persimilis* is pseudoarrhenotokous (Helle *et al.*, 1978).

Amblyseius cucumeris

This predatory mite is not used for biological control. *Amblyseius cucumeris* is pseudoarrhenotokous (Treat, 1965).

Hemipteroseius wormersleyi

This mite was found in association with the hemipteran bug *Ondontopus sexpunctatus* in Ibadan, Nigeria. Although the genetic system of this mite is unknown it is included in the phylogeny as a representative of the Otopheidomenidae, the family which contains the moth ear mite *Dicrocheles phalaenodectes* which is arrhenotokous. For this reason *Hemipteroseius wormersleyi* is coded as pseudoarrhenotokous for the purpose of testing the hypothesis of Schrader and Hughes-Schrader i.e. *Hemipteroseius wormersleyi* is used as a surrogate for *Dicrocheles phalaenodectes* and any inferences made about the position of *Dicrocheles* and the pseudoarrhenotokous clade it represents from the position of

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females which reach an uncolonised habitat patch (e.g. a fresh cowpat) may be able to start a new population by oedipal mating with parthenogenetically produced sons (see Chapter 2).

[95] Including the corpse of a fox (Smith, 1975).

[96] Occasionally including seaweed (Hyatt and Emberson, 1988).

Hemipteroseius wormersleyi in the phylogeny are only as good as the validity of this assumption of surrogacy allows.

Stratiolaelaps miles

This predatory mite is also an agent of biological control. *Stratiolaelaps miles* is arrhenotokous (Walter and Kaplan, 1990) and has a heterochromatised chromosome arm which has been interpreted as the vestiges of the heterochromatised chromosome complement of a pseudoarrhenotokous ancestor (DeJong *et al.*, 1981).

Hypoaspis rosei

This recently described mite is associated with the large Australian cockroaches *Geoscapheus dilatus* and *G. robustus* (Strong and Halliady, 1994). (The individual represented in the phylogeny came from *G. dilatus*.) Since no member of the Dermanyssoidea have been shown to have any genetic system other than arrhenotoky (see chapter 3) it is assumed for the purposes of testing the hypothesis of Schrader and Hughes-Schrader that the genetic system of *Hypoaspis rosei* is arrhenotoky.

Dermanyssus gallinae

This mite, known as the chicken mite or red mite, is the commonest obligatory blood-sucking ectoparasite of both caged and wild birds in Britain, in particular chickens and turkeys. Severe infestations may lead to the death of the birds whilst moderate infestations may only cause loss of condition and reduced egg production (Evans *et al.*, 1961). *D. gallinae* has also been known to bite humans, however, although they are capable of transmitting St. Louis encephalitis to chickens (Smith *et al.*, 1948), it is uncertain whether they are implicated in the spread of this disease to man. For the same reasons as those given for *Hypoaspis rosei* above, *D. gallinae* is considered for the purposes of testing the hypothesis of Schrader and Hughes-Schrader to be arrhenotokous.

Spinturnix myoti

This mite was found on the wing membranes of Daubenton's bats (*Myotis daubentoni*) in where it is assumed to be an ectoparasite. The genetic system of this mite is unknown but it has traditionally been assigned to the superfamily Dermanyssoidea which is characterised by arrhenotoky. The Spinturnicidae have a number of marked adaptations to parasitic life including dorso-ventral flattening, extremely stout legs with well developed claws and viviparity.

Spinturnix plecotinus

This mite was found in association with a Brown Long-Eared Bat (*Plecotus* sp.) from Deecastle, Scotland, and is also assumed to be an ectoparasite. The comments on the

genetic system of *S. myoti* made above also apply to *S. plecotinus*.

Varroa jacobsoni

This mite is an ectoparasite of bees of considerable economic importance which has recently gained entry to the apiaries of England and has rapidly spread North from its introduction site in the Southwest so that only parts of Scotland are now free of it. For the same reasons as those given for *Hypoaspis rosei* and *Dermanyssus gallinae*, for the purposes of testing the hypothesis of Schrader and Hughes-Schrader *V. jacobsoni* is considered to be arrhenotokous.

Characters

A region of 28S ribosomal DNA spanning the D3-D7 regions was amplified and two parts of this region were sequenced in both directions; a area spanning the D3-D5 and a second area within the D7 region. When regions of unalignable and ambiguous sequence were removed sequences were removed a total of 755 base pairs remained. These sequences are shown in appendix 5.1.

Sequence Statistics

Appendices 5.2-5.5 show sequence statistics for all of the sequences represented in the analysis (after removal of unreadable and unalignable sequence). Appendices 5.2 and 5.3 show the base compositions of each of the sequences before and after removal of invariant sites. Appendices 5.4 and 5.5 show the degree of AT bias (Irwin, Kocher and Wilson, 1991), GC-skew (G-C/G+C) and AT-skew (A-T/A+T) (Perna and Kocher, 1995) of each of the sequences, again before and after removal of invariant sites.

The Parasitina (outgroup) have a one base pair insertion with respect to the Dermanyssina (ingroup), and the Spinturnicids a four base pair insertion and a one base pair gap with respect to the rest of the taxa. All of the sequences are AT rich with the greatest degree of bias toward AT in the Parasitina (outgroup).

Stationarity and Time Reversibility

Many distance measures (e.g. uncorrected but not LogDet - see below) assume that the base composition of the sequences remains more or less constant throughout the tree. For this to be the case the data must have the properties of **stationarity** - constancy of the substitution probability matrix throughout the tree, and **time reversibility** - lack of *directionality* in the substitution probability matrix (i.e. the probability of nucleotide a being substituted by nucleotide b should equal the probability of nucleotide b being substituted by nucleotide a for all case in which a and b are different nucleotides A, C, G and T). Stationarity was tested using the test devised by Rzhetsky and Nei (1995). This tests for differences in base composition in different parts of the tree. The Mean pA =

0.276451, mean pG = 0.259167, mean pC = 0.171791, mean pT = 0.292591, I = 35.99 (39 degrees of freedom), P = 0.6079. Stationarity is not rejected i.e. base composition remains constant throughout the tree. Time reversibility was not directly but since a test for base composition differences between different sequences found no difference (see below) it is unlikely that the data are time-irreversible.

Pairwise Sequence Statistics

Base Composition

Pairwise chi-squared tests for were performed for difference in base composition between each pair of sequences. In no case was there a significant difference in base composition between any pair of sequences. The largest difference in base composition was between *Pergamasus septentrionalis* and *Spinturnix myoti* for which chi-squared = 0.268 (1 degree of freedom) which is much less than the threshold for significance at the 5% level (= 3.84). The total chi-squared for all pairwise comparisons = 7.798 (90 degrees of freedom) which again is much lower than the threshold for significance at the 5% level (= 124.3).

Transversion: Transition Ratio

Of 3775 pairwise differences between sequences 1212 (32%) were transversions and 2564 (68%) were transitions. The trasition: transversion ratio = 2.1: 1 which suggests that the sequences are not saturated (see summary to chapter 5 above).

TESTING DATA FOR HIERARCHICAL STRUCTURE

Any data set which contains covariation of character states between different characters will tend to favour some trees over others whatever method of phylogenetic reconstruction is used. Since even randomly generated data will contain some stochastic covariation it is prudent to test a data set for hierarchical structure prior to phylogenetic reconstruction (Swofford *et al.*, 1996). One method for testing for hierarchical structure in a data set is an analysis of the shape of the frequency distribution of tree lengths[97]. Tree length is a property of a particular tree topology given a particular data set. It is the minimum number of character state changes required to account for the observed distribution of character states across the taxa. This value can be calculated for all possible tree topologies (or a random subset of them). Fitch (1979; 1984) observed that the frequency distribution of tree lengths for data sets with little or no hierarchical structure were more or less symmetrical. Hillis and Huelsenbeck (1992) showed that as the amount of hierarchical structure in the data increases the frequency distributions of

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[97] Other methods of testing data for hierarchical structure can be found in Kallersjo *et al.* (1992), Archie (1989; 1993), Lyons-Weiler *et al.* (1996) and Strimmer and von Haeseler (1997).

the tree lengths become more skewed to the left. This implies that if the frequency distribution of tree lengths for a particular data set is significantly skewed to the left then that data set is likely to contain hierarchical structure. This does not necessarily mean the data set will be phylogenetically informative but it does mean that at least some of the covariance in the data is likely to be due to common ancestry and therefore phylogenetic relationships inferred from the data are valid inferences rather than stochastic effects.

The degree of skew of a frequency distribution is measured using the standard g1 statistic. g1 is calculated using a statistic called the third central moment. A central moment is defined as the average of the deviation of all items from the mean, each raised to the power r. The first central moment (r = 1) is always equal to zero, the second central moment (r = 2) is the variance, the third central moment (r = 3) is used to calculate g1 and the fourth central moment is used to calculate another statistic (g2 or kurtosis) which also describes the shape of the frequency distribution (Balanda and MacGillivray, 1988). g1 is equal to the third central moment divided by the cube of the standard deviation.

g1 is positive for right skewed frequency distributions, negative for left skewed distributions and zero for symmetrical distributions. g1 statistics can be tested for statistically significant departure from 0 and this can be used as the basis for a statistical test for hierarchical structure in a data set. Care must be exercised since it is not clear exactly how g1 is related to the degree of hierarchical structure in the data and different parts of a tree may make different contributions to g1. For example the data set may be highly structured with respect to the groupings in one clade but entirely random with respect to those in another. Although a phylogenetic reconstruction method may fully resolve both of these clades this resolution would be a valid phylogenetic inference in one clade but a stochastic effect in the other. Hillis (1991) has suggested a procedure for detecting which groupings are most responsible for the hierarchical structure in a data set by calculation of g1 following successive restriction of the sample space of trees.

In order to test a data set for hierarchical structure a single tailed t test can be used to test whether the frequency distribution of tree lengths is the significantly skewed to the left . The null hypothesis is that g1 statistic is not significantly less than zero (H_0: g1 = 0, H_1: g1<0). $t = (g1 - g1)/s_{g1}$ where g1 is calculated from the data, g1 is the value of g1 predicted by the null hypothesis (= 0) and s_{g1} is the standard error of the g1 statistic (= the square root of 6/n, where n = the sample size for n>150). The appropriate number of degrees of freedom for calculating P is ∞ (Sokal and Rohlf, 1995). For small data sets an exhaustive search can be performed which will give the tree lengths for all possible tree topologies but for large data sets a subset of all possible tree topologies can be used. The number of tree topologies sampled must be large since for small sample sizes g1 will tend to underestimate the actual degree of skew since the value of g1 calculated from the sample can never be greater than the square root of the sample size (Wallis, Matalas and Slack, 1974).

For the 28S data 10000 tree topologies were randomly generated using PAUP 3.1. (Swofford, 1993). g1 = -1.919. n = 10000. s_{g1} = 0.0245. t = 78.36. P<<0.001 i.e. the probability is much less than one in a thousand that the g1 statistic calculated from the data has been sampled from an actual distribution of tree lengths that is not skewed to the left i.e. it is highly likely that the data set contains hierarchical structure and that it can be used to make valid phylogenetic inferences.

PHYLOGENETIC RECONSTRUCTION

Algorithmic Methods

Algorithmic methods of phylogenetic reconstruction consist of all those methods in which a similarity or distance matrix is converted into a phylogenetic tree by the operation of some particular algorithm. Before such an algorithm can be used a similarity or distance matrix must therefore be constructed and there are a number of different ways in which this can be done. These are comprehensively reviewed by Swofford *et al.* (1996) and only those two which were used in this analysis (uncorrected distances and LogDet distances) are discussed here.

Uncorrected Distance

Pairwise uncorrected distances ("p") also called dissimilarity distances ("D") (Kumar, Tamura and Nei, 1993) are simply the number of differences (nucleotide substitutions) between two sequences divided by the total number of sites available for pairwise comparison . This is essentially the same as the branch length between the two sequences in an optimal phylogenetic tree of the two taxa. Uncorrected distances assume equal base composition throughout the tree and no rate heterogeneity among sites.

LogDet Distances

LogDet (Lockhart *et al.*, 1994; Steel, 1994) or paralinear (Lake, 1994) distances allow for differences in base composition in different parts of the tree but in common with uncorrected distances they assume no rate heterogeneity among sites (Barry and Hartigan, 1987). The method for calculating LogDet distances can be found in Swofford *et al.* (1996).

Correcting Distance Measures to Take Account of Rate Heterogeneity Among Sites

Distance measures which assume no rate heterogeneity among sites will underestimate the actual number of substitutions in data sets in which there is rate heterogeneity (Golding, 1983). Since all data sets can be expected to contain some degree of rate heterogeneity among sites and both of the distances measures used here and described above assume no rate heterogeneity among sites it may be necessary to correct the distance measures to allow for this factor. There are two approaches which have been

taken to this problem, one based on continuous distributions of substitution rates among sites and another based on discrete distributions.

Continuous Distribution of Substitution Rates

Some types of distance measure can be modified to follow a continuous **gamma distribution** however this is inappropriate for the two distance measures used in this analysis and will therefore not be discussed in detail here.[98] (A discrete gamma distribution of substitution rate was used to construct maximum likelihood trees. This is discussed in more detail in the maximum likelihood section below.)

Discrete Distribution of Substitution Rates

A second approach to the problem of rate heterogeneity among sites, and the one used in this analysis, is the **invariable sites model**. According to this model a certain fraction of constant sites are assumed to be incapable of being substituted (due, for example, to functional constraints on the molecule) and are removed from the data matrix prior to calculation of the distances. The remainder of the sites are assumed to vary at the same rate (Churchill, von Haeseler and Navidi, 1992; Hasegawa, Kishino and Yano, 1985; Reeves, 1992; Sidow, Nguyen and Speed, 1992). This is a special case of a discrete distribution in which the number of categories = 2 and the rate of one of the categories = 0. Other, more complicated discrete distributions with larger numbers of categories with different non-zero rates could be used if this was indicated by the data.[99]

The number of sites invariant for each of the four nucleotides A, C, G and T which are removed = Ø N / 4 where Ø = the desired proportion of invariable sites and N = the total number of sites (Waddell (1995) cited in Swofford *et al.* (1996)). Two values for Ø were used in the analyses below. Initially the value of Ø was set at 0.2. This was a purely arbitrary value used in an initial neighbour joining analysis chosen only because some value for this parameter had to be entered. Subsequent analyses used Ø = 0.65. This value was estimated from the data using a maximum likelihood estimator based on the tree topology given by the initial neighbour joining analysis (see figure 5.1 below) and since it was estimated from the data this probably represents a more realistic value of Ø for this data set. If base frequencies are unequal then it is necessary to remove $\pi k \emptyset N$ constant sites with base k, where πk is the frequency of base k for each of the four bases A, C, G

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[98] For more details on these methods see Jin and Nei (1990), Tamura and Nei (Tamura and Nei, 1993), Waddell and Steel (1995), Lewis and Swofford unpublished (cited in Swofford *et al.* (1996)), Swofford (1996) Kumar *et al.* (1993).

[99] Gu *et al.* (1995) and Waddell and Penny (Waddell and Penny, 1996) advocate an invariant plus gamma model in which some sites are invariable whilst the rates of the remainder are gamma distributed. (This is the approach used to calculate the maximum likelihood trees (see below) in which a proportion of sites are assumed to be invariable while the rates of the remainder are distributed according to a discrete gamma distribution with four rate classes so that the number of categories = 5 and the rate of one of the categories = 0.) Swofford *et al.* (1996) recommend classifying sites into a number of rate classes, applying the LogDet transformation to each and summing to obtain a final estimate of distance.

and T. If base composition is not constant throughout the tree, or if constant sites have a different composition than variable sites (which will occur if the data are time irreversible) then the base frequencies used to calculate the correction for rate heterogeneity should be estimated from the constant sites alone. Since the data are stationary and there was no significant difference in base composition between any pair of sequences (see above) this refinement was considered unnecessary.

Algorithmic Methods for Ultrametric Trees

An ultrametric tree is one in which each three taxon triplet satisfies the three-point metric condition. This states that of the three pairwise distances between three taxa two are equal and at least as large as the third. This means that every internal node (including the root) is equidistant from all its descendant terminal nodes i.e. cluster analysis assumes an exact molecular clock (Williams, 1992a; Williams, 1992b). Although the rate of evolutionary change need not be linear in time the relationship between rate of evolutionary change and time must be the same in all parts of the tree. Superimposed sequence changes which decrease the apparent rate of nucleotide substitution will not disrupt the ultrametric property since this effect will be equal in all parts of the tree (Swofford *et al.*, 1996).

Cluster Analysis

The term cluster analysis refers to a group of algorithms in which ultrametric trees are constructed from similarity or distance data (Sneath and Sokal, 1973). Cluster methods are prone to error if the data are not ultrametric and therefore should not be used for data in which the rate of evolution is considered likely to vary across the tree. Even if the underlying substitution rate is constant, sequence data are unlikely to be ultrametric unless the number of nucleotides sequenced is large enough to eliminate the stochastic effects of sampling error[100]. If the data can be considered to be nearly ultrametric, cluster analysis can be more efficient (require less data to achieve the same probability of inferring the correct tree) than other methods (Swofford *et al.*, 1996), however, it is generally unreasonable to assume that a data set will be ultrametric. The degree to which the assumption of ultrametricity can be violated without cluster methods becoming inconsistent (more likely to favour an incorrect tree as more data are added) has been investigated by Colless (1970). One of the major recommendations for the use of cluster analysis is the relative speed of these methods, however, neighbour joining (below) is nearly as fast, not subject to the assumption of ultrametricity and often more efficient under a molecular clock model (Sourdis and Krimbas, 1987) at least when substitution rates are low. The most common clustering methods are UPGMA (unweighted pair group method using arithmetic averages) and WPGMA (weighted PGMA). Since there is no

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[100] Genome hybridisation experiments come close to satisfying this condition but are subject to large measurement errors (Felsenstein, 1987).

reason to believe that the 28S data are ultrametric, it is considered inappropriate to use cluster techniques to infer phylogenetic relationships from this data set.

Algorithmic Methods for Non-Ultrametric Trees

Since data in are not usually ultrametric there has been considerable interest in designing algorithmic methods which can be used on non-ultrametric data. There have been three distinct approaches to this problem. The most successful approach to this problem has been to transform the non-ultrametric matrix to an ultrametric matrix and then perform cluster analysis[101] . Methods which fall into this category include the transformed distance method (Li, 1981), the present day ancestor method (Klotz and Blanken, 1981) and the neighbour joining method (Saitou and Nei, 1987).

Neighbour Joining

Of all of the algorithmic methods the most widely used is the neighbour joining method. This method is related to cluster analysis but removes the requirement that the data be ultrametric. The data must however be additive. An additive tree is one in which each four taxon quartet satisfies the four-point metric condition (Buneman, 1971). This states that of the three sums of pairwise distances $d_{ij} + d_{kl}$ where $i \neq j \neq k \neq l$ between four taxa two are equal and at least as large as the third. This means that the distance between any two taxa is equal to the sum of the lengths of the branches joining them[102]. Data containing superimposed substitutions will not be additive but there are methods available for transforming such data to additive data by correcting for multiple hits. It is important to perform such transformations when using methods such as neighbour joining which are sensitive to non-additivity.

For each terminal node the sum of the distance between that node and each of the other nodes is calculated. This is the net divergence for that node from all other taxa (r_i)· This value is then used to transform the data matrix by replacing each element with a rate corrected distance. The rate corrected pairwise distance between nodes i and j (M_{ij}) is calculated as:

$$M_{ij} = d_{ij} - (r_i + r_j) / (N - 2)$$

Where d_{ij} = the uncorrected distance between nodes i and j, r_i is the net divergence of node i from all other nodes, r_j is the net divergence of node j from all other nodes and N is the number of terminal nodes in the matrix.

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[101] This is by no means the only approach that has been taken to the problem. See Farris (Farris, 1972), Sattah and Tversky (Sattah and Tversky, 1977), and Fitch (Fitch, 1981) for alternatives.

[102] Branch lengths represent evolutionary distances between pairs of taxa at least one of which is a hypothetical ancestor.

A new node (u) is defined uniting the two terminal nodes separated by the smallest rate corrected distance (i and j)[103]. The length of the branch[104] from node u to node i (v_{iu}) is calculated as:

$$v_{iu} = d_{ij} / 2 + (r_i - r_j) / (2 (N-2))$$

The length of the branch from node u to node j (v_{ju}) is calculated as:

$$v_{ju} = d_{ij} - v_{iu}$$

The distance from node u to each other terminal node except nodes i and j (node k) are calculated as:

$$d_{ku} = (d_{ik} + d_{jk} - d_{ij}) / 2$$

The distance to nodes i and j are removed from the data matrix and N is decreased by 1. If more than two nodes remain the entire process is repeated otherwise the tree is fully resolved and the length of the final branch joining the two remaining nodes is simply the uncorrected distance between these nodes.

Since neighbour joining is much faster than most optimisation methods of tree construction (see below) it is often used to produce an initial tree from which the parameters needed for a more thorough search using other methods are estimated. It is rarely used unsupported by other methods as the only way of reaching a final tree.

A number of neighbour joining bootstrap trees were constructed using PAUP* Version 4.0.d52 for Macintosh (Swofford, 1996) in order to give maximum likelihood estimates of various parameters for the evolutionary model used in to construct a maximum likelihood tree (see below). These neighbour joining trees are shown in figures 5.1-5.5.

In the first analysis gaps were treated as missing data, the two spinturnicids were included, LogDet distances were used and the proportion of sites assumed to be invariable was arbitrarily set at 0.2 (an unrealistically low estimate). 100 bootstrap replicates were performed. Figure 5.1 shows the 50% majority rule consensus of these 100 replicates.

The arrhenotokes form a clade with a bootstrap support of 73%. The haplodiploid taxa (i.e. arrhenotokes + pseudoarrhenotokes) form a clade with a bootstrap support of 62%. The Phytoseiidae (pseudoarrhenotokes) form a clade with bootstrap support of 68% but

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[103] Since rate corrected distances are always negative this means choosing the *most negative* value of M_{ij}.

[104] Since neighbour joining represents the data as an additive tree the algorithm may produce negative branch lengths if the data are non-additive. The modification of Kuhner and Felsenstein (1994) sets negative length branches equal to zero and transfers the difference to the adjacent branch so that the total distance between a pair of terminal nodes is unaffected. This has no effect on tree topology.

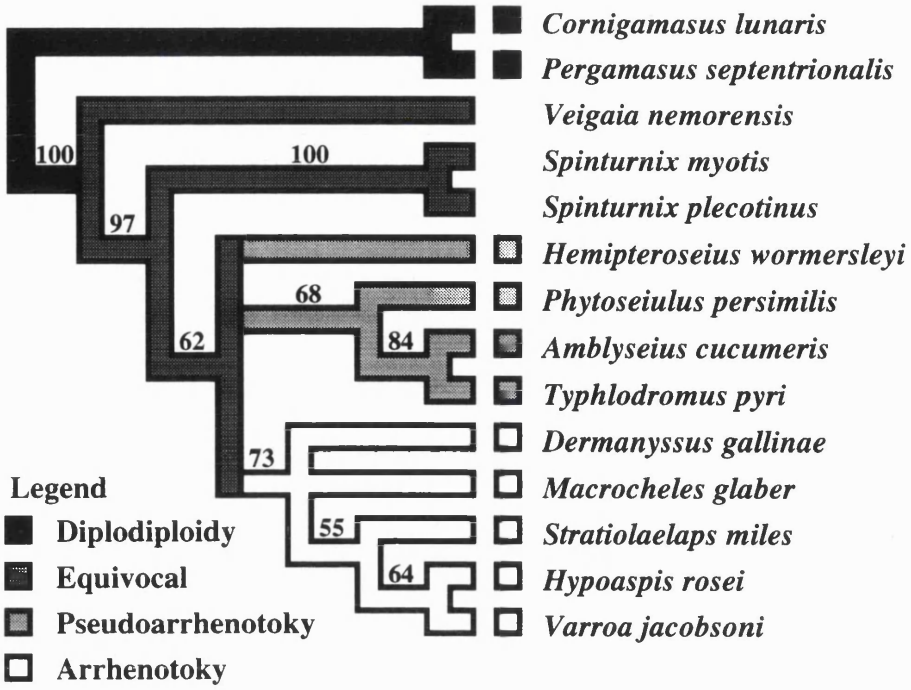

Figure 5.1: 50% majority rule consensus of 100 neighbour joining bootstrap replicates

Figures above resolved nodes represent the bootstrap support for those nodes. Gaps were treated as missing data and the two spinturnicids were included. The distance measure used to construct this tree was the LogDet distance. The proportion of sites assumed to be invariable was 0.2.

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the relationship between the Phytoseiidae, *Hemipteroseius wormersleyi* (also pseudoarrhenotokous) and the arrhenotokes is unresolved i.e. it is not known which of these three taxa are sister groups. If the two pseudoarrhenotokous groups (Phytoseiidae + *Hemipteroseius wormersleyi*) were sister groups then the pseudoarrhenotokes would form a clade which would be the sister group of the arrhenotokes. This would not necessarily support the hypothesis of Schrader and Hughes-Schrader since there would be no evidence that the arrhenotokes arose from a pseudoarrhenotokous ancestor rather than directly from a zygogenetic diplodiploid one but it would not rule out this hypothesis either since it would be possible that the arrhenotokes arose from a pseudoarrhenotoke at the base of the pseudoarrhenotokous clade and that a pseudoarrhenotokous taxon basal to this one had not been sampled (either because it had not been selected for inclusion in the phylogeny or because it had gone extinct). If either pseudoarrhenotokous clade were sister group to the arrhenotokes however, then this would support the hypothesis of Schrader and Hughes-Schrader since the monophyletic arrhenotokes would have arisen within a paraphyletic pseudoarrhenotokous assemblage. Since the sister group

relationships within these three taxa are not resolved this tree provides no evidence either for or against the hypothesis of Schrader and Hughes-Schrader.

A second neighbour joining bootstrap tree was constructed using a different distance measure, the uncorrected distance. The proportion of sites assumed to be invariable was 0.65 (a much better estimate than in the previous analysis). This was calculated from the data using maximum likelihood and the topology of the tree shown in figure 5.1. The analysis parameters were otherwise unaltered from those used in the first analysis. Figure 5.2 shows the 50% majority rule consensus of these 100 bootstrap replicates.

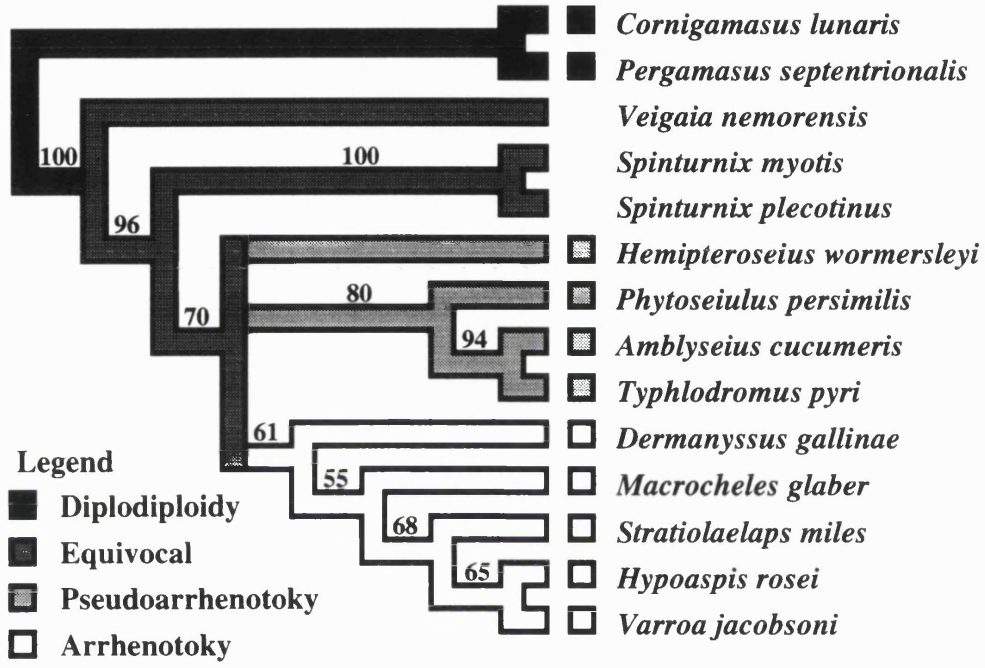

Figure 5.2: 50% majority rule consensus of 100 neighbour joining bootstrap replicates.

Figures above resolved nodes represent the bootstrap support for those nodes. Gaps were treated as missing data and the two spinturnicids were included. The distance measure used to construct this tree was the uncorrected distance. The proportion of sites assumed to be invariable was 0.65.

The crucial node remains unresolved but the unresolved node within the arrhenotokous clade is now resolved. The topology of the rest of the tree remains unaltered i.e. this tree is not in conflict with the first although it has more resolution. Most of the bootstrap values are a little higher e.g. the bootstrap support for the Phytoseiidae has increased from 68% to 80% and that for the haplodiploid clade has increased from 62% to 70% but the bootstrap support for the arrhenotokous clade has decreased from 73% to 61%. Since only 100 bootstrap replicates were performed and the differences are not large it is difficult to know whether these differences in bootstrap values represent genuine

differences in the degree of support for these nodes or are simply due to stochastic processes. The statistical properties of bootstrap values are poorly understood.

A third neighbour joining bootstrap tree was constructed. LogDet distances were used as in the first analysis, but this time with the more realistic value of 0.65 for the proportion of sites assumed to be invariable from the second analysis. 1000 bootstrap replicates were performed. The analysis parameters were otherwise unaltered from those in the second analysis. Figure 5.3 shows the 50% majority rule consensus of these 1000 bootstrap replicates.

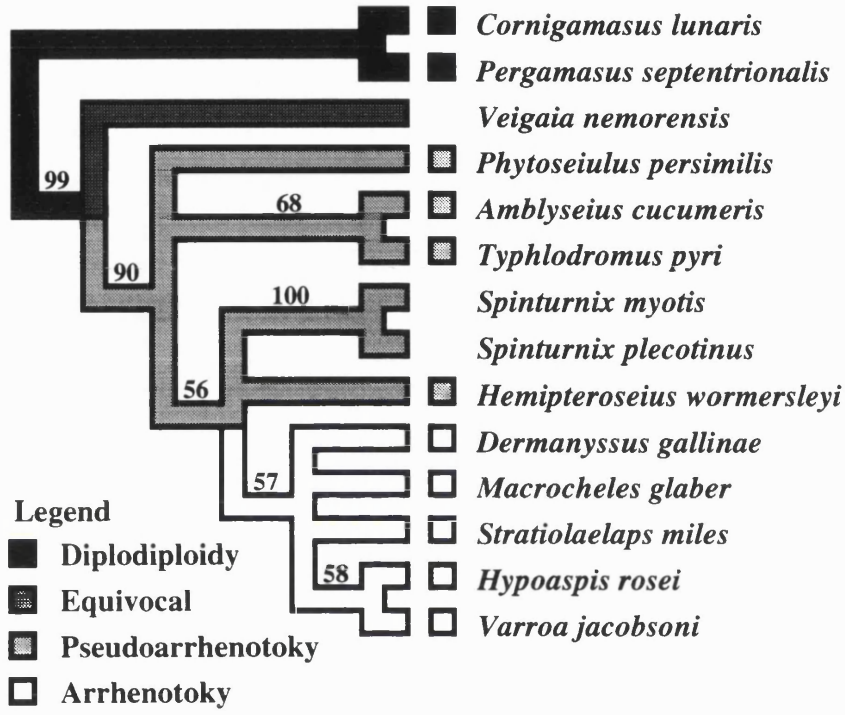

Figure 5.3: 50% majority rule consensus of 1000 neighbour joining bootstrap replicates.

Figures above resolved nodes represent the bootstrap support for those nodes. Gaps were treated as missing data and the two spinturnicids were included. The distance measure used to construct this tree was the LogDet distance. The proportion of sites assumed to be invariable was 0.65.

This tree is poorly resolved compared with the first two. Since this analysis combines the supposedly more realistic aspects of each the first two this may suggest that the resolution in the those trees is misleading (despite the fact that they are not in conflict). Although this tree is less resolved it is does resolve the node crucial for a test of the hypothesis of Schrader and Hughes-Schrader which neither of the previous trees did. The arrhenotokous clade which has a bootstrap support of 57% (which is rather low), arises *within* a parahaploid assemblage which is paraphyletic and includes the two

spinturnicids[105]. The hypothesis of Schrader and Hughes-Schrader hinges on the sister group relationship of the arrhenotokes. For the hypothesis to be true the sister group of the arrhenotokes must be a pseudoarrhenotokous clade but also in order to demonstrate that arrhenotoky is the derived condition and pseudoarrhenotoky the primitive one this clade consisting of a clade arrhenotokes which are the sister group of a clade of pseudoarrhenotokes must itself be the sister group of another clade of pseudoarrhenotokes[106]. Since these conditions are both satisfied in this tree it can be said to support the hypothesis of Schrader and Hughes-Schrader. The bootstrap support for the nodes crucial to this test are 56% for the node uniting the arrhenotokes with their pseudoarrhenotokous sister group (*Hemipteroseius wormersleyi* (plus the spinturnicids)) and 90% for the node uniting this clade with a further pseudoarrhenotokous clade (in this case *two* further pseudoarrhenotokous clades ([*Phytoseiulus persimilis*] and [*Amblyseius cucumeris* + *Typhlodromus pyri*]), the relationships between which are unresolved, but the conclusions remain the same). Relationships within the arrhenotokes are poorly resolved but this has no effect on the test of the hypothesis of Schrader and Hughes-Schrader. This tree predicts that the spinturnicids are pseudoarrhenotokous. An elucidation of the genetic systems of the spinturnicids would be a good test of the hypothesis of Schrader and Hughes-Schrader particularly since this phylogeny removes the spinturnicids from their traditional place within the Dermanyssoidea, a group characterised by arrhenotoky.

A fourth neighbour joining bootstrap tree was constructed. This time gaps were treated as a fifth base. 100 bootstrap replicates were performed. The analysis parameters were otherwise unaltered from those in the third analysis. Figure 5.4 shows the 50% majority rule consensus of these 100 bootstrap replicates.

Although this tree differs from the previous tree only in the position of *Hemipteroseius wormersleyi* the node crucial to the test of the hypothesis of Schrader and Hughes-Schrader is now unresolved. The arrhenotoke clade has a bootstrap support of 52% and again the relationships within this clade are poorly resolved. The haplodiploid clade has a bootstrap proportion of 87% and again includes the Spinturnicids. These are the sister group of the arrhenotokes (the bootstrap support for this node is 51%) but the sister group

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[105] Since the genetic systems of the two Spinturnicids are unknown their position in the tree cannot effect a test of the hypothesis of Schrader and Hughes-Schrader (or indeed any other hypothesis regarding the evolution of genetic systems). They may therefore appear anywhere in the tree. Even when we take into account the observation that they must mate in order to produce viable offspring then this only excludes them from being arrhenotokes and the hypothesis of Schrader and Hughes-Schrader only requires that they do not appear within the arrhenotokous clade but they may appear anywhere else in the tree. The position of these taxa can therefore never be used to support the hypothesis of Schrader and Hughes-Schrader although it may be used against it.

[106] If it were the sister group of another clade of arrhenotokes then pseudoarrhenotoky would be a condition secondarily derived from arrhenotoky, if it were the sister group of a clade of ancestral zygogenetic diplodiploids then it would be unknown whether the arrhenotokous clade derived from a pseudoarrhenotokous ancestor as is required by the hypothesis of Schrader and Hughes-Schrader or directly from a zygogenetic diplodiploid ancestor - this is the case in the first two neighbour joining trees. This means that at least two pseudoarrhenotokous taxa must be included in the phylogeny in order to have any chance at all of providing support for the hypothesis of Schrader and Hughes-Schrader.

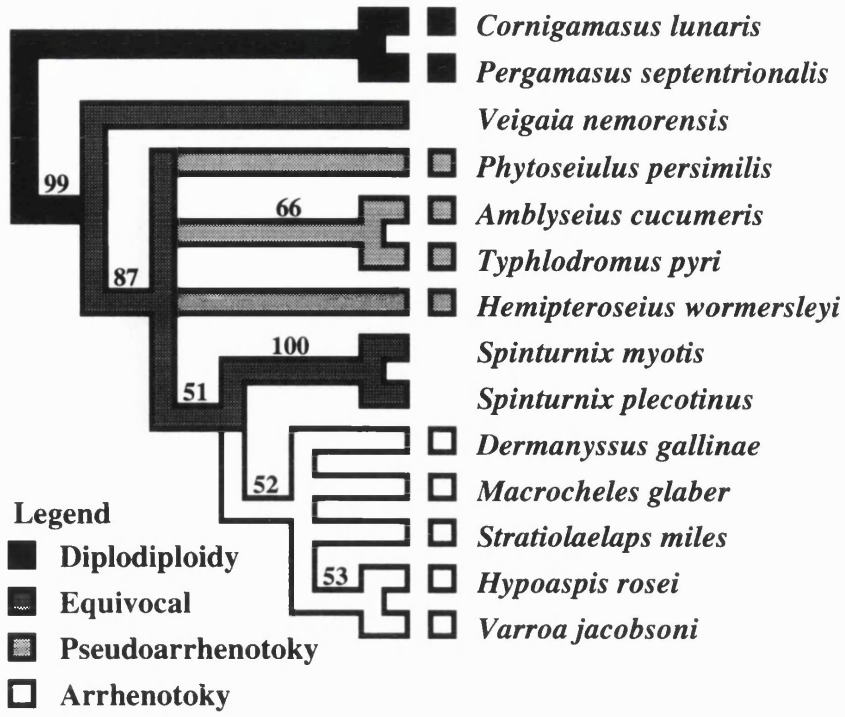

Figure 5.4: 50% majority rule consensus of 100 neighbour joining bootstrap replicates.

Figures above resolved nodes represent the bootstrap support for those nodes. Gaps were treated as a fifth base and the two spinturnicids were included. The distance measure used to construct this tree was the LogDet distance. The proportion of sites assumed to be invariable was 0.65.

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of this clade, crucial to a test of the hypothesis of Schrader, is unresolved. The sister group of the arrhenotokes (plus the spinturnicids) could be [*Phytoseiulus persimilis*], [*Amblyseius cucumeris* + *Typhlodromus pyri*], [*Hemipteroseius wormersleyi*] or a clade formed by the combination of any two or , crucially, all three of these clades. Any of these possibilities except the last would provide support for the hypothesis of Schrader and Hughes-Schrader but since the tree permits the possibility that the sister group of the arrhenotokes is a clade formed by all of the pseudoarrhenotokes and this in turn permits the possibility that the arrhenotokes arose directly from a zygogenetic diplodiploid ancestor rather from a pseudoarrhenotokous intermediate, this tree cannot be said to provide support for the hypothesis of Schrader and Hughes-Schrader. Equally, however, it does not provide any evidence *against* this hypothesis. This tree also fails to resolve the monophyly of the Phytoseiidae.

A fifth neighbour joining bootstrap tree was constructed with the two spinturnicids excluded. The analysis parameters were otherwise unaltered from the previous analysis. Figure 5.5 shows the 50% majority rule consensus of these 100 bootstrap replicates.

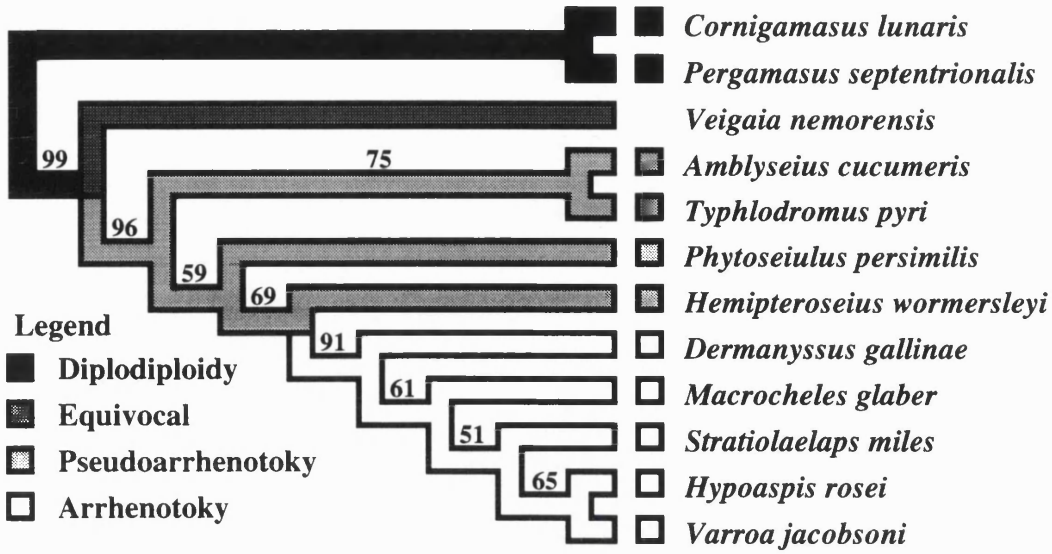

Figure 5.5: 50% majority rule consensus of 100 neighbour joining bootstrap replicates.

Figures above resolved nodes represent the bootstrap support for those nodes. Gaps were treated as a fifth base and the two spinturnicids were excluded. The distance measure used to construct this tree was the LogDet distance. The proportion of sites assumed to be invariable was 0.65.

Removal of the Spinturnicids from the analysis appears to have a radical effect on the remainder of the tree which is now fully resolved. This is the only neighbour joining tree which contains no unresolved nodes. This is not merely because removal of the spinturnicids removes the source of unresolved nodes because all other trees contain unresolved nodes which do not involve the Spinturnicids, including the tree which differs from this one only in the inclusion of the spinturnicids in the distance matrix from which it was constructed. This means that the removal of the spinturnicids has genuinely increased the resolution in the remainder of the tree. The bootstrap support for most nodes has also increased, for example the bootstrap support for the arrhenotokes has increased from 52% to 91% simply by removing the spinturnicids and the relationships within this clade are now fully resolved. The bootstrap support for the haplodiploid clade has increased from 87% to 96%.

The reason for removal of the spinturnicids is that they are on the longest branch of the tree and this branch is in fact substantially longer than any other. This suggests that the rate of evolution (in this region of 28S rDNA at least) is greater in the lineage that gave rise to the spinturnicids than in the rest of the tree. This is also suggested by the fact that the two spinturnicids share a three base pair insertion which is not possessed by any of the other taxa represented. (The only other insertion in the data is a one base pair insertion found in the two outgroup taxa.) In phylogenetic reconstruction in general, but

particularly in methods such as neighbour joining which are based on pairwise distances, the presence of long branches can worsen the effects of systematic error and the effect of removing the spinturnicids from this data set provides a dramatic illustration of this principle.

Another way of dealing a long branch is to split it by including a taxa which are likely to attach to the tree at some point along this branch. Since we do not know which families of the Dermanyssoidea are most closely related to the Spinturnicidae it would be difficult to make appropriate suggestions as to which other family within this superfamily would be most likely attach to the tree along this branch but it may be worth including a representative of another genus within the Spinturnicidae in an attempt to split up this long branch. *Spinturnix* is a derived genus within the Spinturnicidae (Rudnick, 1960) and the inclusion of a more primitive genus such as one of those confined to the old world where the family is thought to have originated (e.g. *Ancystropus*, *Meristaspis*, *Eyndhovia* or *Paraperglischrus*) may have the effect of splitting up this long branch.

This tree provides unequivocal support for the hypothesis of Schrader and Hughes-Schrader. The arrhenotokes arise within a paraphyletic pseudoarrhenotokous assemblage. The sister group of the arrhenotokes is *Hemipteroseius wormersleyi* (bootstrap support = 69%). The sister group of the arrhenotokes + *Hemipteroseius wormersleyi* is *Phytoseiulus persimilis* (bootstrap support = 59%) and the sister group of the arrhenotokes + *Hemipteroseius wormersleyi* + *Phytoseiulus persimilis* is a clade consisting of *Amblyseius cucumeris* + *Typhlodromus wormersleyi* (bootstrap support = 96%). In this tree the Phytoseiidae is paraphyletic.

In summary, none of the neighbour joining trees conflict with the hypothesis of Schrader and Hughes-Schrader and two of the five provide support for this hypothesis. Furthermore the two trees which support the hypothesis are those with arguably the most realistic assumptions. Nevertheless the neighbour joining algorithm suffers from a number of problems (for example branch length estimates from neighbour joining are not, in general, optimal for the criterion of minimum evolution (Swofford *et al.*, 1996)) and its chief advantage is its speed. For this reason it is usually unwise to make inferences from neighbour joining trees alone and unsupported by any other method of phylogenetic reconstruction.

The Position of the Root

Although the trees shown in figure 5.1-5.5 are unrooted they are displayed as if the root of the tree were between the Parasitina and the Dermanyssina i.e. these two cohorts are assumed to be monophyletic groups which is the traditional taxonomic view of the

group[107]. However, since this is an *assumption* of the test of the hypothesis of Schrader and Hughes Schrader and not an *inference* made from the data it is important to make this assumption explicit and to explore the consequences of violation of this assumption. Two possibilities will be considered for the position of the root, outside the ingroup (i.e. between the ingroup and the outgroup (which would both be monophyletic) or within the outgroup (which would be paraphyletic with respect to the ingroup) or within the ingroup (which would be paraphyletic with respect to the outgroup).

There are a number of male reproductive characters which support monophyly of Dermanyssina, Parasitina and [Dermanyssina + Parasitina]. Male reproductive characters which are synapomorphic for the Dermanyssina include podospermy and a spermatodactyl. Male reproductive synapomorphies in support of Parasitina include a spermatotreme and filiform sperm. Male reproductive synapomorphies in support of [Dermanyssina + Parasitina] include ribbon type sperm. These characters are discussed further in appendix 5.6.

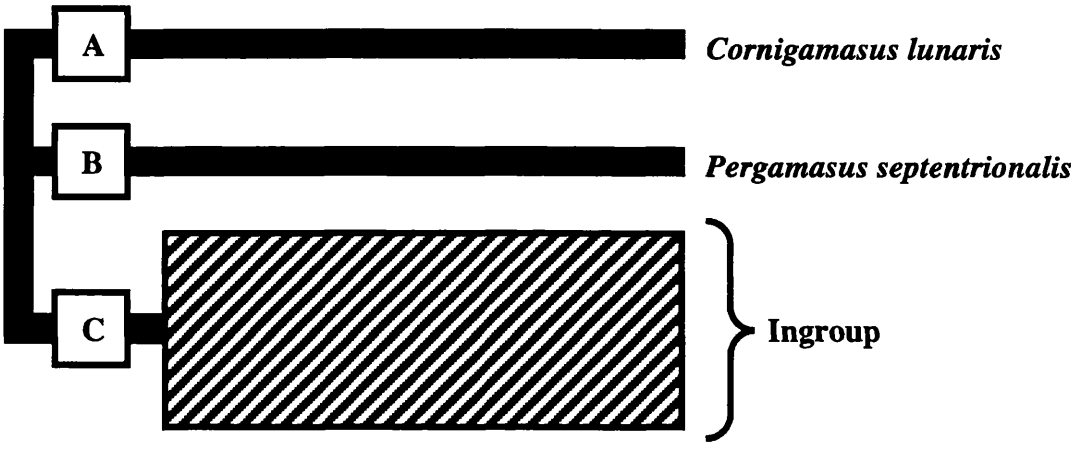

Figure 5.6: The three positions of the root which are compatible with a monophyletic ingroup

Root Outside the Ingroup

Figure 5.6 shows the three positions of the root which are compatible with a monophyletic Dermanyssina. If the root were at positions A or B the Parasitina would be paraphyletic (if A were the root then the sister taxon of the Dermanyssina would be *Pergamasus septentrionalis*, if B were the root then the sister taxon of the Dermanyssina would be *Cornigamasus lunaris*). If the root were at position C then both Parasitina and Dermanyssina would be monophyletic. This is the way the trees have been represented in figures 5.1-5.5. None of these hypotheses would be counter to the hypothesis of Schrader

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[107] For example see Berlese (1906), Bregetova (1956), Evans and Till (1965; 1966; 1979), Bhattacharyya (1963), Radovsky (1969), Athias-Henriot (1967; 1979), Holzmann (1969), Micherdzinski (1969), Samsinak and Dusbabek (1971), Krantz (1978), Hyatt (1980), Johnston (1982), Krantz and Ainscough (1990), Gilyarov and Bregetova (1977), and Johnston in Norton *et al.* (1993).

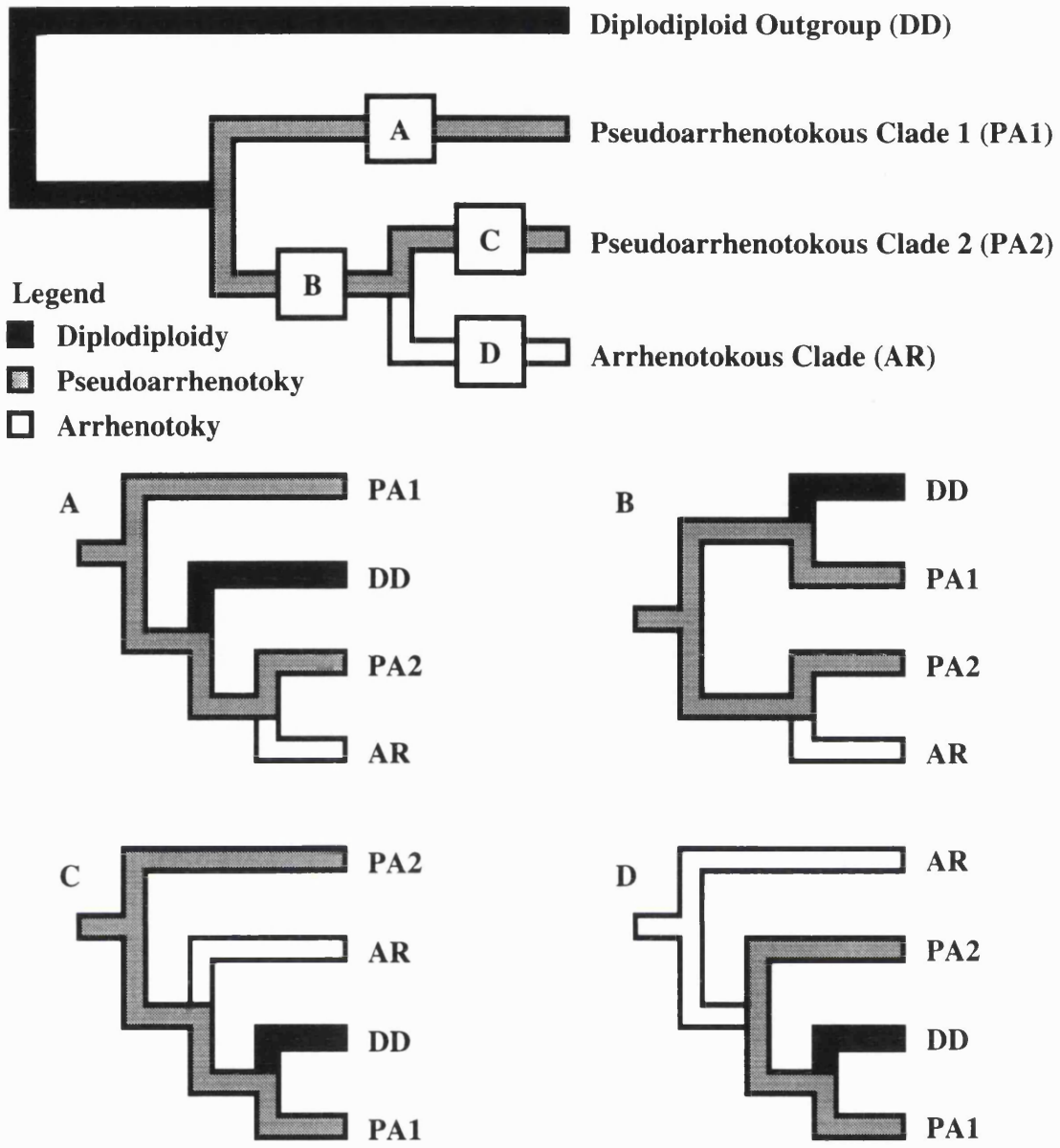

Figure 5.7: Four positions of the root which are incompatible with a monophyletic ingroup and their effect on the hypothesis of Schrader and Hughes-Schrader.

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and Hughes-Schrader and therefore the monophyly or paraphyly of the Parasitina has no bearing on any test of the hypothesis of Schrader and Hughes-Schrader.

Root Within the Ingroup

Any tree which supports the hypothesis of Schrader and Hughes-Schrader must have at least four elements:

- A diplodiploid outgroup
- An arrhenotokous clade
- A pseudoarrhenotokous clade as sister group to the arrhenotokous clade

- A second pseudoarrhenotokous clade as sister group to the arrhenotokes + the first pseudoarrhenotokous clade.

Figure 5.7 shows these elements and the various places in which the root could be placed on this tree within the ingroup. Placing the root at positions A, B or C maintains support for the hypothesis of Schrader and Hughes-Schrader since in each of these trees the arrhenotokous clade arises from a pseudoarrhenotokous ancestor rather than directly from a pseudoarrhenotokous ancestor, however, in each of these trees there is also a reversal to the ancestral diplodiploid zygogenetic state from pseudoarrhenotoky. If, however, the root were placed at position D then this would be counter to the hypothesis of Schrader and Hughes-Schrader since pseudoarrhenotoky would have arisen from arrhenotoky rather than the other way around. This tree also a reversal to ancestral diplodiploid zygogenesis from pseudoarrhenotoky. Position D, however, is the least likely of all positions for the root (except for *within* the arrhenotokous clade) since it is the furthest from the position of the root according to the traditional taxonomic view.

In summary, placement of the root anywhere on the tree, except for the place where it is least likely to be found (i.e. at the base of or within the arrhenotokes) will not effect the conclusion that these data support the hypothesis of Schrader and Hughes-Schrader. Placement of the root *within* the four groups represented at the terminal nodes of the trees in figure 5.7 will yield trees equivalent to those given for placement of the root at the base of these groups except that these groups will be paraphyletic with respect to the rest of the taxa in the tree.

Optimality Methods

Optimality methods for inferring phylogeny depend not on the operation of an algorithm but on selecting the tree or set of trees from the set of all possible trees which maximises the value of some particular optimisation criterion. Comparing the optimisation criterion of all possible trees (exhaustive searching) can be computationally demanding, particularly for large data sets and therefore a number of heuristic search methods have been developed which select a subset of all the possible trees which is likely to contain the tree which maximises the optimisation criterion. These usually operate in the following way:

1 A tree or set of trees is selected from the set of all possible trees as a starting point[108].

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[108] The commonest method for selecting the starting tree and the one used by the program PAUP (Swofford, 1993; Swofford, 1996) below is known as stepwise addition. As each taxon is added to the tree, the optimisation criterion is evaluated for each position that the new taxon could be added to the tree and it is added at the position which maximises the value of the optimisation criterion. There are various methods for deciding the order in which to add the taxa (e.g. Felsenstein (1993), Farris (1970)) but the method used here as implemented by PAUP starts with the three taxon tree which maximises the optimisation criterion (in this case parsimony) and then adds all remaining taxa at all possible branches on the tree and chooses taxon branch combination which requires the smallest increase in tree length. Stepwise addition is known as a 'greedy' algorithm since it maximises short term increases in optimality at the possible expense of long term increases i.e. the placement of a taxon which may be best for the taxa currently on the tree may

2 The optimisation criteria for the tree or trees selected in step 1 is calculated.
3 The tree or trees in step 1 are altered in some way.
4 The optimality criteria for the new trees are calculated.
5 Those trees that have higher optimality criteria than the trees from which they were derived are selected as the starting point and the process begins again at step 3.
6 When the optimality criteria no longer continue to increase a local optimum has been reached.

This process, known as hill climbing, can only find local optima. There are many different methods for altering trees at step 3 (Charleston, 1995; Reeves, 1995). These alterations to the tree are called perturbations and there is much argument in the literature over the relative benefits of using each of them[109]. There is also a large literature discussing the problem of finding the global optimum, or of assessing the probability that a local optimum is indeed also the global optimum[110]. Alternatives to hill climbing differ only in the criteria by which trees are accepted at step 5. Hill climbing only accepts trees which have a higher optimisation criterion than the tree from which they were derived but, for example in simulated annealing (Van Laarhoven and Aarts, 1987), a tree may also be accepted with a certain probability if it has a lower *optimisation* criterion and this probability may vary over time. In the Great Deluge Method (Dueck, 1990; Dueck and Scheuer, 1990) the tree is accepted if the optimisation criterion reaches a particular threshold and this threshold increases slowly with time. Other alternatives to hill climbing can be found in Glover (1989) and Swofford *et al.* (1996).

Maximum Parsimony

Maximum parsimony methods of phylogenetic reconstruction select trees which minimise the number of character state changes on the tree (tree length) (Camin and Sokal, 1965; Sober, 1989). All maximum parsimony analyses were performed using PAUP version 3.1 for Macintosh (Swofford, 1993). A branch and bound search gave four maximally parsimonious trees (figure 5.8) (Tree length = 230.) A branch and bound search is equivalent to an exhaustive search but ignores groups of trees which it calculates can only possibly contain suboptimal trees (Hendy and Penny, 1982). It will always

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become suboptimal upon the addition of more taxa. Greedy algorithms are prone to entrapment in local optima. The major alternative to stepwise addition is star decomposition (also a greedy algorithm) in which the terminal taxa of a star phylogeny are sequentially united saving the tree that scores best for the optimality criterion at each step (Adachi and Hasegawa, 1992; Saitou, 1990; Yang, 1995).

[109] Examples include nearest neighbour interchange (NNI) in which two neighbouring portions of the tree are swapped with one another, subtree pruning and regrafting (SPR) in which part of the tree is removed and placed somewhere else and tree bisection and reconnection (TBR) in which the tree is divided into two parts which are then reconnected at different points.

[110] The preferred strategy is to use multiple starting trees to see whether all of these lead to the same optimum which is therefore presumably the global optimum, or whether they lead to a number of different trees which represent a number of different local optima. Opinions differ on the methods for choosing the starting trees. A the set of starting trees can be derived by performing stepwise addition on a set of random sequences for the addition of taxa. A more extreme approach consists of using random trees rather than random addition sequences but Swofford *et al.* (1996) consider this approach to be less effective since it would yield starting trees which are still a long way from the optima.

recover the globally optimal tree but will usually operate much faster than an exhaustive search (but much slower than a heuristic search, the branch and bound search took 12 minutes and 4 seconds, as opposed to 49-62 seconds for the heuristic searches - see below). Since it does not consider all possible trees it does not generate a frequency distribution of tree lengths but in all other respects can be considered equivalent to an exhaustive search. Gaps were treated as missing data. Heuristic searches were also run in order to evaluate their use in assessing the value of a number of a posteriori reweighting schemes (see below). Starting trees were obtained by stepwise addition using the addition sequence which maximised parsimony at each step (see above). Only minimal trees were kept throughout (in both stepwise addition and branch swapping) and only one tree was held at each step. Three types of branch swapping were used; nearest neighbour interchange (NNI), subtree pruning and regrafting (SPR) and tree bisection and reconnection (TBR). All three methods recovered the globally optimal trees but since NNI was the fastest (49 seconds as opposed to 51 seconds for SPR and 62 seconds for TBR) only this was used for constructing trees based on various reweighting schemes (see below). Removal of the two Spinturnicids which are on the longest branch of the tree did not effect the topology of the rest of the trees but did increase the bootstrap proportions of the other nodes (see below).

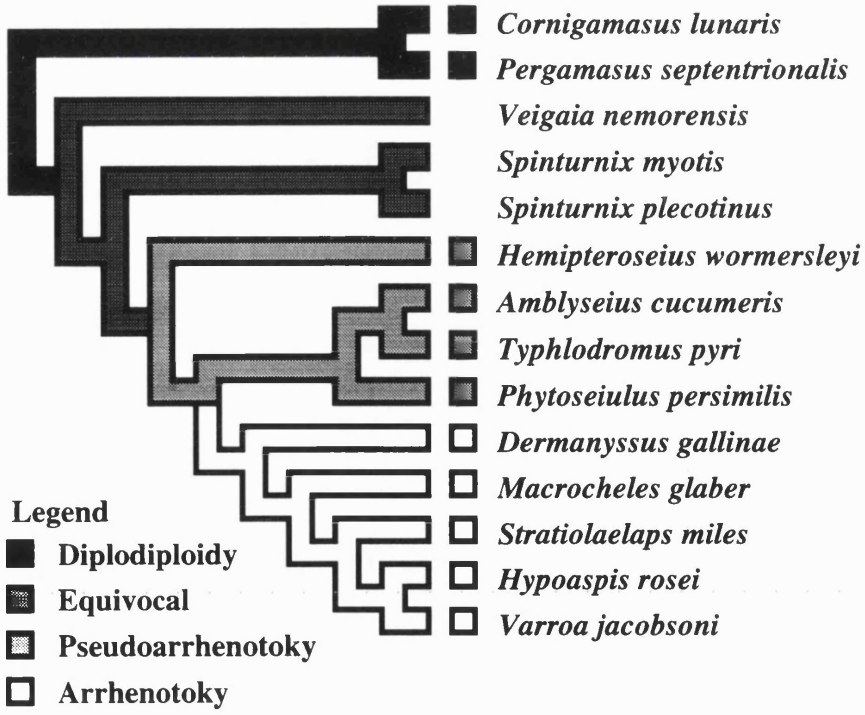

Figure 5.8a: Maximum Parsimony Tree #1
(See text for details)

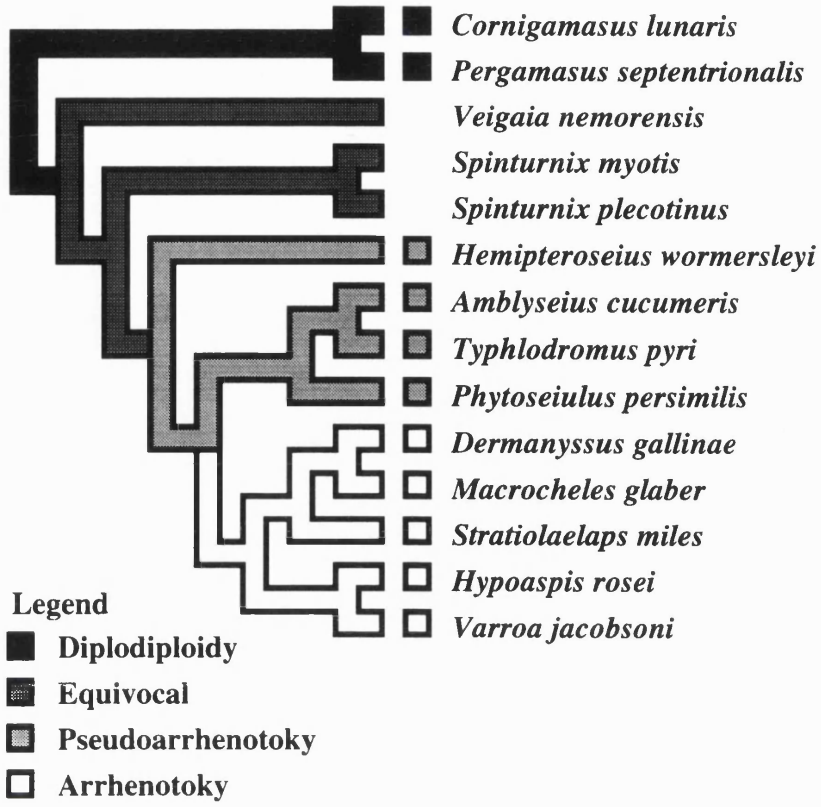

Figure 5.8b: Maximum Parsimony Tree #2
(See text for details)

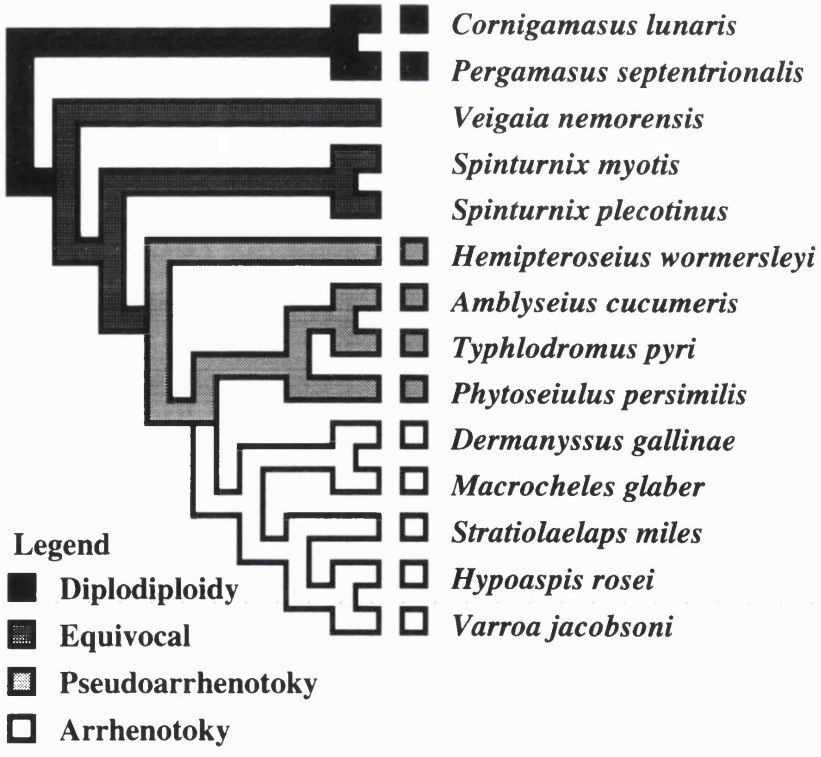

Figure 5.8c: Maximum Parsimony Tree #3
(See text for details)

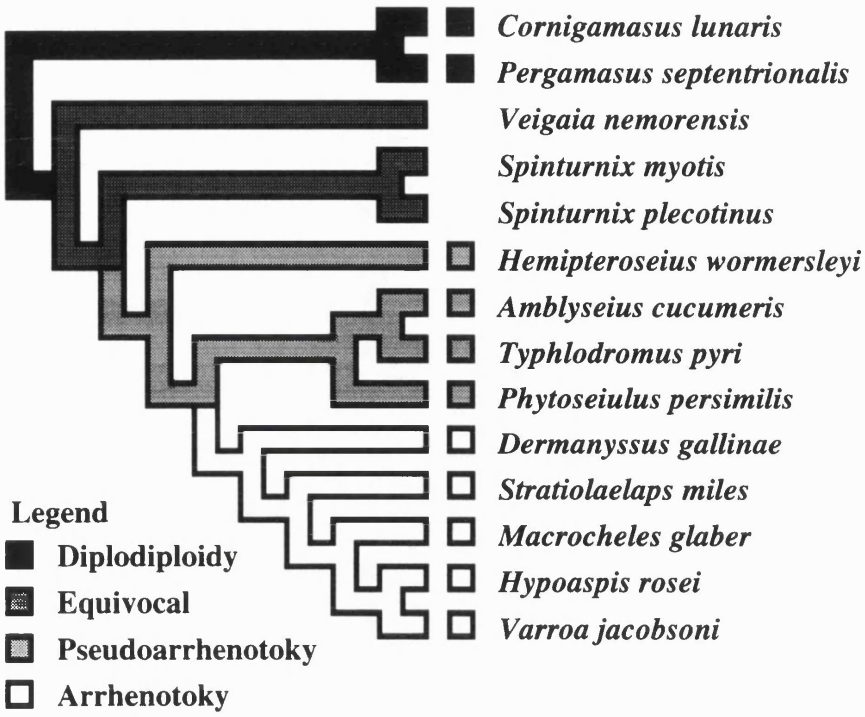

Figure 5.8d: Maximum Parsimony Tree #4
(See text for details)

An examination of a strict consensus of these four trees (figure 5.9) indicates the unresolved portion of the tree consists of a clade containing all of the arrhenotokes. The position of this clade within the tree is stable, only the relationships within it are unresolved and therefore the tree can be used to test the hypothesis of Schrader and Hughes-Schrader (see below). Since this clade contains *Stratiolaelaps miles*, an arrhenotoke which has a heterochromatised chromosome arm which has been interpreted as the possible remnant of the heterochromatised chromosomal complement of a pseudoarrhenotokous ancestor (DeJong *et al.*, 1981) and may therefore represent an intermediate stage in the evolution of arrhenotoky from pseudoarrhenotoky it would be a pity to leave this clade unresolved.

Methods for Choosing Between Equally Parsimonious Trees

Filtering

The size of a set of maximally parsimonious trees can sometimes be reduced by removing all polytomous trees for which a more highly resolved compatible tree exists. This does not resolve any conflict between the trees but chooses between trees which are not in conflict but differ in the amount of resolution they offer, those trees which contain the greatest resolution. The size of the set of maximally parsimonious trees obtained from the 28S rDNA data set cannot be reduced by filtering indicating that these four trees are in genuine conflict i.e. they cannot all be simultaneously true.

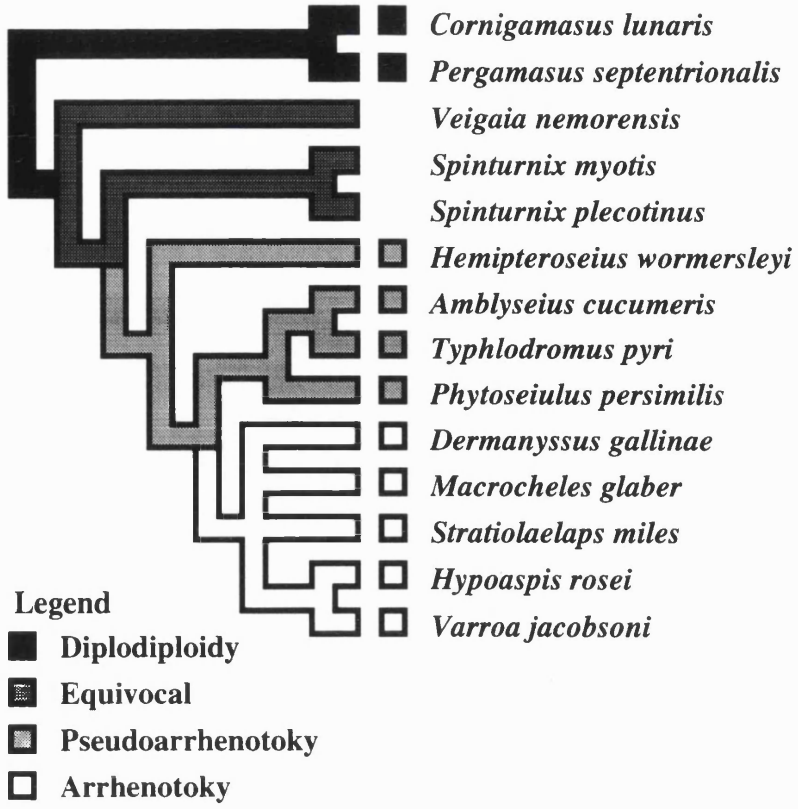

Figure 5.9: The strict consensus of Maximum Parsimony Trees #1, #2, #3 and #4 (figures 5.8a-d)
(See text for details)

Reweighting

Nine different a posteriori reweighting schemes were tried to see if reweighting the data could decrease the size of the set of maximally parsimonious trees. In all cases a single

tree was obtained however, this was not always the same tree. Reweighting according to the maximum, mean and minimum values of the Consistency Index (CI) all gave tree #2 whereas reweighting according to the maximum, mean and minimum values of the Retention Index (RI) and Rescaled Consistency Index (RC = CI x RI) all gave tree #1. There is no consensus on which index is most appropriate for a posteriori reweighting schemes and therefore there is no way to choose between trees #1 and #2. The fact that two of these indices favour tree #1 whilst only one favours tree #2 reflects the ways that the indices are calculated rather than the strength of support for particular phylogenetic hypotheses. The fact that reweighting always gave either tree #1 or #2 may indicate that the data give more support for trees #1 and #2 than for trees #3 and #4, however, a strict consensus of trees #1 and #2 is no more resolved than a strict consensus of all four trees. An Adams consensus (Adams, 1972) of trees #1 and #2 is more resolved than an Adam's consensus of all four trees, which could be interpreted as an increase in resolution resulting from excluding trees #3 and #4 but this tree contains groups which are not

present in any of the four original trees. A semi-strict consensus is no more resolved than a strict consensus for either trees #1, #2, #3 and #4 or trees #1 and #2. In conclusion, a posteriori reweighting has little, if any, effect on resolution in this data set and therefore a strict consensus of the four most parsimonious trees remains the most appropriate summary of the phylogenetic information contain within the data according to parsimony based methods.

Parsimony Bootstrapping

Two parsimony bootstrap analyses were performed, one with the spinturnicids included and gaps treated as missing data (figure 5.10) and a second with spinturnicids removed and gaps treated as a fifth base (figure 5.11).

As in the neighbour joining trees the removal of the spinturnicids seems to have a radical effect on amount of resolution in the tree. (It seems unlikely that the difference in resolution is due to the way in which gaps are treated since there are so few gaps in the data set.) Removal of the spinturnicids adds three nodes to the tree and increases the bootstrap proportions of most other nodes. Crucially the tree which includes the spinturnicids does not resolve the relationships necessary to test the hypothesis of Schrader and Hughes-Schrader whereas removal of the spinturnicids yields a tree which is in support of this hypothesis. The arrhenotokes group with a bootstrap support of 73%. The sister group of the arrhenotokes is the pseudoarrhenotoke *Hemipteroseius wormersleyi* with a bootstrap support of 67% and the sister group of this clade is either *Phytoseiulus persimilis*, [*Amblyseius cucumeris* + *Typhlodromus pyri*] or a clade comprising both of these. Since all of these three taxa are pseudoarrhenotokous phytoseiids, the lack of resolution at this point in the tree does not effect the test of the hypothesis of Schrader and Hughes-Schrader. The bootstrap support for the clade containing all of the haplodiploid taxa has a bootstrap support of 96%.

Maximum Likelihood

Likelihood is a statistical measure of how probable the observed data are given a particular tree and model of evolution (Cavalli-Sforza and Edwards, 1967; Goldman, 1990). This can be assessed for all possible trees (or a subset of trees in a heuristic search), and the tree which has the greatest likelihood chosen. Likelihood differs from parsimony in a number of important ways. Firstly likelihood requires a model of evolution[111] and for that reason has been applied realistically only to molecular data[112]

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[111] To say that parsimony methods do *not* require a model of evolution is not to say that they do not rely on assumptions however, there is much disagreement as to what these assumptions are and indeed whether this even matters.

[112] Nucleotide (Felsenstein, 1981a; Felsenstein, 1993; Hasegawa and Yano, 1984; Kishino and Hasegawa, 1989; Swofford, 1996) and amino acid (Adachi and Hasegawa, 1992; Kishino, Miyata and Hasegawa, 1990) sequences, gene frequencies (Felsenstein, 1981b) and RFLPs (Felsenstein, 1992).

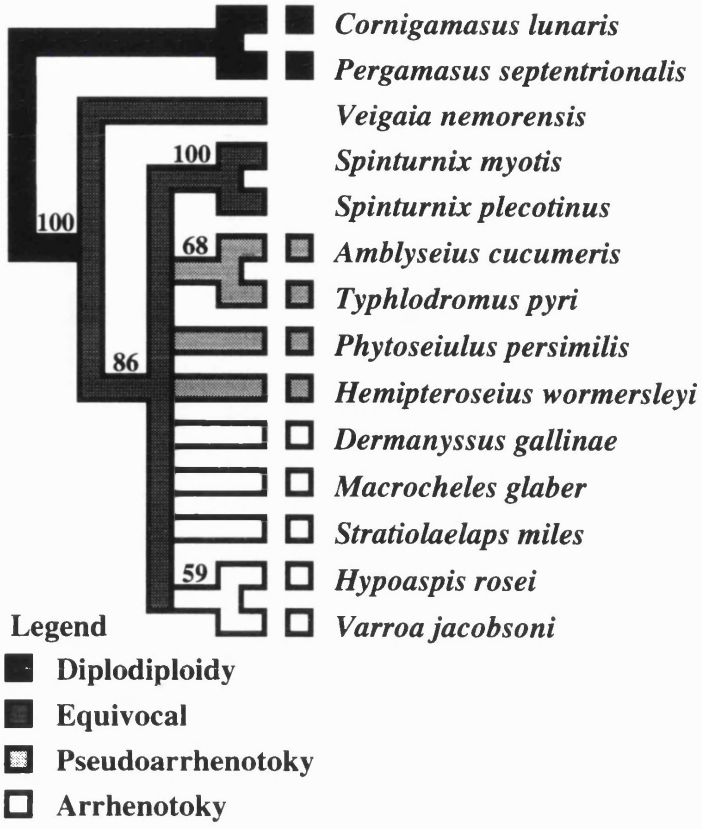

Figure 5.10: 50% majority rule consensus of 100 maximum parsimony bootstrap replicates.

Figures above resolved nodes represent the bootstrap support for those nodes. Gaps were treated as missing data and the two spinturnicids were included. Trees were constructed using a heuristic search with starting trees constructed by stepwise addition with taxa added in the order in which they appear in the data matrix (as given in appendix 5.1 with the exception that duplicated sequences were only included once[113]), One tree held at each step during stepwise addition and branch swapping performed using the tree bisection and reconnection algorithm.

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since the genetic model underlying morphological evolution is rarely known in any detail. The model may be fully defined or alternatively some of the parameters of the model may be estimated from the data. Secondly, likelihood based methods were originally developed to overcome an undesirable property of parsimony based methods called inconsistency (Felsenstein, 1978). Under certain circumstances, particularly (although not exclusively (Hendy and Penny, 1989)) when rates of evolution differ in different parts of the tree parsimony based methods can give greater support of the *wrong* tree as more characters are added. Such behaviour is said to be inconsistent. Maximum likelihood was

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[113] With the exception of the placement of *Stratiolaelaps miles* between *Hemipteroseius wormersleyi* and *Hypoaspis rosei*, this is in alphabetical order.

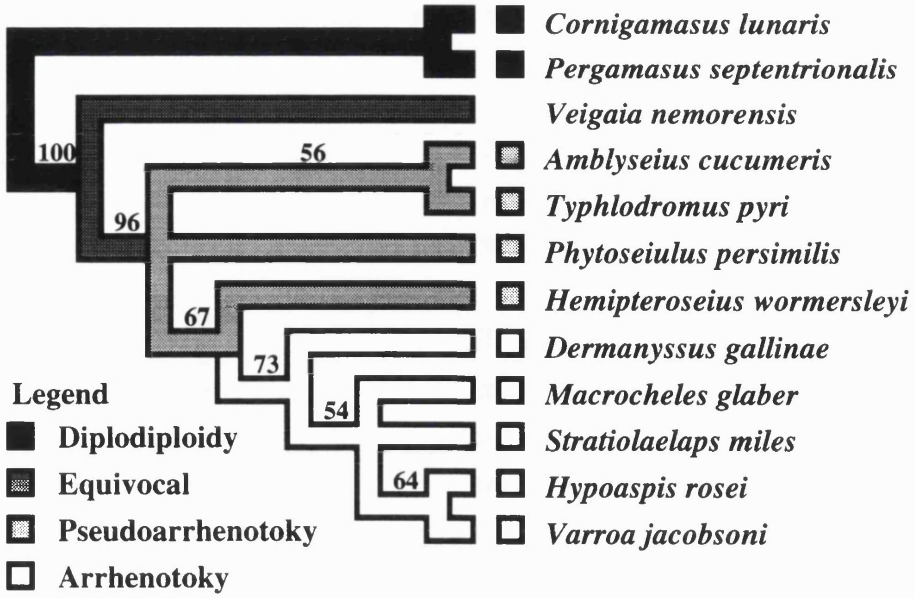

Figure 5.11: 50% majority rule consensus of 100 maximum parsimony bootstrap replicates.

Figures above resolved nodes represent the bootstrap support for those nodes. Gaps were treated as a fifth base and the two spinturnicids were excluded. Trees were constructed using a heuristic search with starting trees constructed by stepwise addition with taxa added in the order in which they appear in the data matrix. One tree was held at each step during stepwise addition and branch swapping performed using the tree bisection and reconnection algorithm.

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invented in order to provide a method which would be consistent under all circumstances, i.e. as more characters are analysed likelihood should always be more likely (or at least never be less likely) to recover the true tree (even at the possible expense of requiring more characters to achieve the same level of resolution as parsimony).[114]

Three maximum likelihood analyses were performed using PAUP* Portable version 4.0.0d52 for Unix (Swofford, 1996). In the first analysis the two spinturnicids were included, gaps were treated as missing data and the topology of the neighbour joining tree shown in figure 5.1 was used to estimate the parameters used in the model of sequence evolution. The maximum likelihood estimate for the proportion of invariable sites estimated from the neighbour joining tree in figure 5.1 = 0.65 (The observed proportion of constant sites was 0.82.). Rates (for variable sites) were assumed to follow a discrete gamma distribution (Yang, 1994) in which the gamma distribution is divided into four

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[114] Another approach to the problem of inconsistency of parsimony based methods is so called corrected parsimony in which the data are corrected for unobserved substitutions using a Hadamard conjugation prior to the operation of parsimony based methods. Parsimony analysis of this new data set will be consistent provided certain assumptions are true (e.g. no among site heterogeneity) (Steel, Hendy and Penny, 1993).

rate categories separated by boundaries in the distribution which give each category an equal probability. The mean rate of each category is then used to represent all of the rates within that category. This prevents the necessity of obtaining likelihoods by integrating over the entire gamma distribution which is computationally intensive (Yang, 1993). (The maximum likelihood estimate for the shape parameter of the gamma distribution estimated from the neighbour joining tree in figure 5.1 = 0.74). The HKY85 (Hasegawa-Kishino-Yano) model of sequence evolution (Hasegawa *et al.*, 1985) was used since it is able to accommodate unequal base frequencies. This is a two parameter model i.e. substitutions are of two types, transitions and transversions. (The maximum likelihood estimate of the transition: transversion ratio estimated from the neighbour joining tree in figure 5.1 = 2.61). This model assumes time reversibility and stationarity (see above). A heuristic search was performed. The starting trees were obtained via stepwise addition, taxa being added in the order in which they appear in the data matrix (as given in appendix 5.1 with the exception that duplicated sequences were only included once). 1 tree was held at each step during stepwise addition and tree bisection and reconnection (TBR) branch swapping was performed. The best 20 best trees were saved. Figure 5.12 shows the best tree. Figure 5.13 shows the strict consensus of these 20 best trees. A semi-strict consensus of these trees is no more resolved than the strict consensus.

These 20 trees range in Ln likelihood values from -2224.06 to -2225.53. Since Ln likelihoods are continuous variables unlike tree lengths in maximum parsimony (which are discrete variables) we are unlikely to get multiple equally optimal trees. There will always be some difference in the likelihoods between the very best tree and apparently slightly less likely trees, but this slight difference may not be statistically significant. Any trees which are less likely than the most likely tree to a degree which is not statistically significant should be considered members of a set of equally likely trees. The strict consensus of this entire set of most likely trees will therefore be the best (conservative) summary of the valid phylogenetic inferences which can be made from the data. If the values for Ln likelihoods at the extremes of the range of these 20 trees are not statistically significantly different from each other then these 20 trees will all appear in the set of most likely trees and the most fully resolved phylogeny which is genuinely supported by the data can be no more resolved than that shown in figure 5.13. Indeed it may be even less so if many more trees even less likely than these 20 are not statistically significantly less likely than the most likely tree.

A method for testing whether two Ln likelihoods are statistically significantly different from each other is the Ln likelihood ratio test. This simple test calculates the test statistic δ (Goldman, 1993) (= G of Sokal and Rohlf (1995)). $\delta = 2 (\text{Ln } L_1 - \text{Ln } L_2)$ and is approximately χ^2 distributed with q degrees of freedom, where q = the difference in the number of free parameters between the null and alternative hypotheses (Huelsenbeck and Rannala, 1997). In this case $\delta = 2.95$ and q=1 (since the alternative hypothesis has one

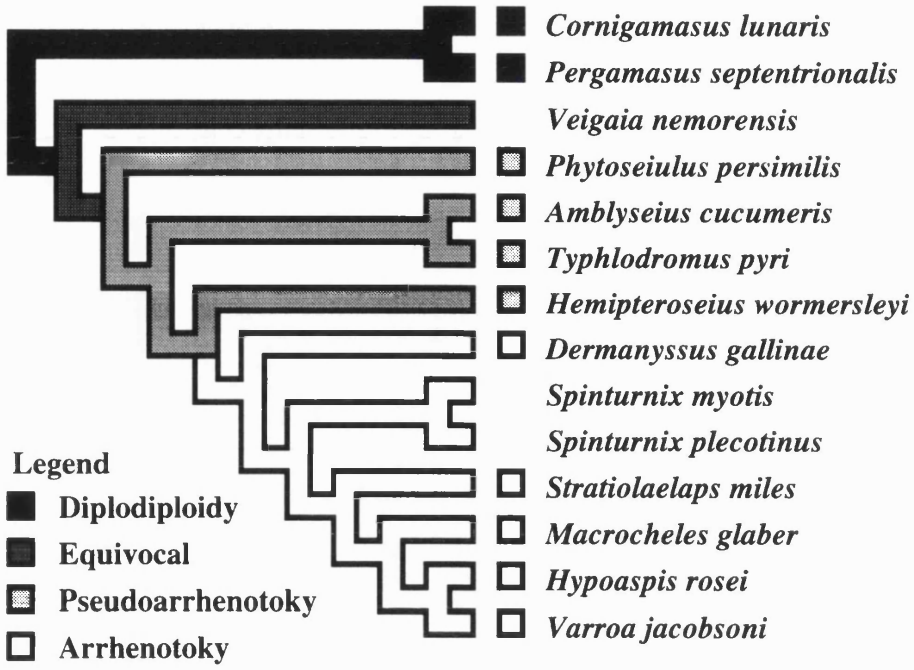

Figure 5.12: Maximum likelihood tree with spinturnicids included and gaps treated as missing data.

For details of model parameters see main text.

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more free parameter (topology[115]) than the null hypothesis). This value is slightly less than the threshold for significance at the 5% level (3.84) but grater than the 10% threshold (2.71) so although this indicates that the most likely tree is no more likely than the 20th most likely the threshold for significance is being approached and it will be crossed when the Ln likelihood drops below -2225.98. Any tree with a likelihood greater than this should be included in the set of most likely trees.

A second maximum likelihood analysis was performed using the topology of the maximum likelihood tree shown in figure 5.13 to estimate the parameters used in the model of sequence evolution. This time the spinturnicids were removed from the analysis and 100 bootstrap replicates were performed. Gaps were again treated as missing data. The proportion of sites assumed to be invariable = 0.67 (The observed proportion of constant sites was 0.85.). Rates (for variable sites) were assumed to follow a discrete gamma distribution with shape parameter = 0.72 and 4 rate categories. The HKY85 model of sequence evolution was used and the transition: transversion ratio was estimated to be 2.51. The heuristic search parameters were the same as those used in the first

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[115] There is a great deal of controversy over whether topology is a genuine parameter. This is a complex issue which will be discussed no further here, however, it should be kept in mind when interpreting the results of likelihood ratio tests which compare different hypotheses of tree topology.

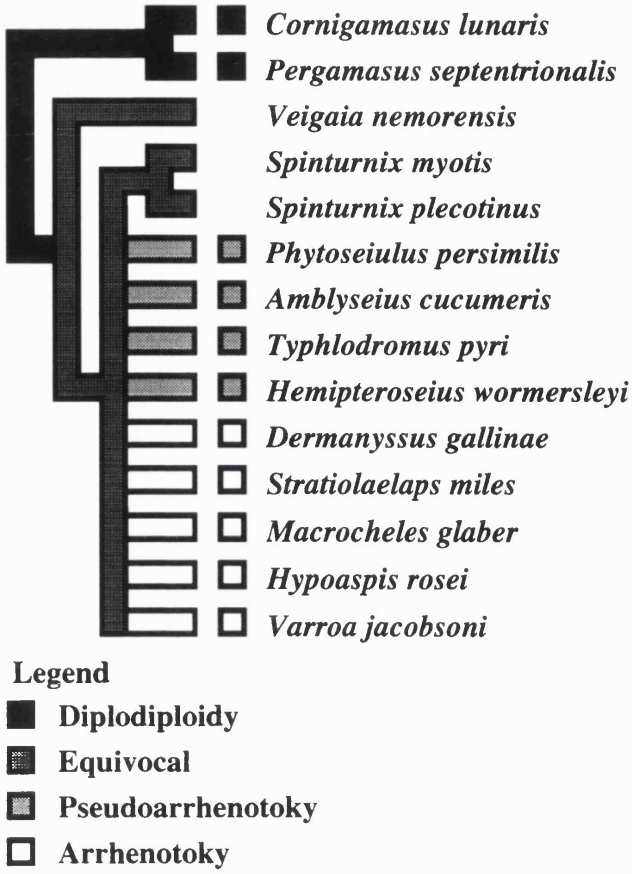

Figure 5.13: Strict Consensus of 20 best maximum likelihood trees with spinturnicids included and gaps treated as missing data.

For details of model parameters see main text.

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analysis (see above). Figure 5.14 shows the 50% majority rule consensus of the 100 bootstrap replicates.

A third maximum likelihood analysis was performed. Likelihoods were calculated individually for each character using topology of the parsimony tree in figure 5.11 and the characters were weighted according to these likelihoods. Gaps were treated as a fifth base, the spinturnicids were excluded and the model parameters were estimated from the parsimony tree in figure 5.11. The proportion of sites assumed to be invariable = 0.67 (The observed proportion of constant sites was 0.85.). Rates (for variable sites) were assumed to follow a discrete gamma distribution with shape parameter = 0.72 and 4 rate categories. The HKY85 model of sequence evolution was used and the transition: transversion ratio was estimated to be 2.51.

In order to test whether the global optimum had been found the addition sequence in the stepwise addition step of the heuristic search (see above) was randomised and 10

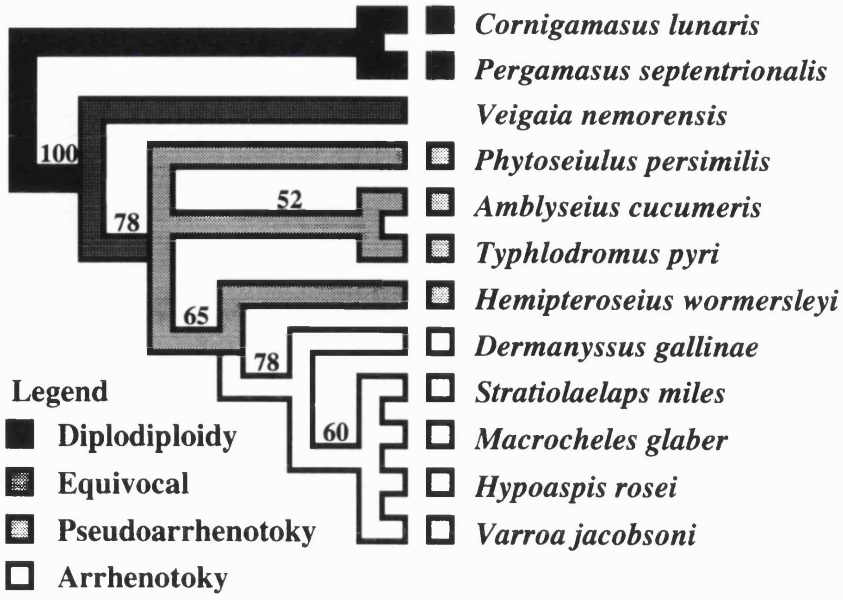

Figure 5.14: 50% majority rule consensus of 100 maximum likelihood bootstrap replicates with spinturnicids removed and gaps treated as missing data.

Figures above resolved nodes represent the bootstrap support for those nodes. For details of model parameters see main text.

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replicates were performed. All 10 of these found the same optimal tree (Ln likelihood = -1958.24) indicating that the global optimum had probably been found. This tree was identical to that in figure 5.12 but with the spinturnicids removed.

In contrast to the neighbour joining tree, removal of the spinturnicids has no effect on the degree of resolution of the maximum likelihood tree. Treatment of gaps as missing data or as a fifth base similarly has no effect. All of the maximum likelihood trees provide support for the hypothesis of Schrader and Hughes-Schrader. The maximum likelihood bootstrap tree is very similar to the parsimony bootstrap tree with the spinturnicids removed (figure 5.11). The only difference between them is that the parsimony tree groups *Hypoaspis rosei* with *Varroa jacobsoni* while the likelihood tree leaves this node unresolved. The arrhenotokes group with a bootstrap support of 78%. The sister group of the arrhenotokes is the pseudoarrhenotoke *Hemipteroseius wormersleyi* with a bootstrap support of 65% and the sister group of this clade is either *Phytoseiulus persimilis*, [*Amblyseius cucumeris* + *Typhlodromus pyri*] or a clade comprising both of these. Since all of these three taxa are pseudoarrhenotokous phytoseiids, the lack of resolution at this point in the tree does not effect the test of the hypothesis of Schrader and Hughes-Schrader. The bootstrap support for the clade containing all of the haplodiploid taxa has a bootstrap support of 78%. In contrast to the neighbour joining and parsimony trees the maximum likelihood tree which includes the spinturnicids puts them in their traditional place within the Dermanyssoidea and therefore predicts that they are arrhenotokous.

COMPARISON OF DIFFERENT METHODS OF PHYLOGENETIC RECONSTRUCTION

Kim (1993) suggested that an appropriate way to test the reliability of a tree is to see whether is produced by a number of different methods of phylogenetic reconstruction. Since different methods are subject to different biases and sources of error any tree which can be derived from a number of different methods should be free of such problems. All of the methods described above gave the same pattern of results. In no case did any tree contradict the hypothesis of Schrader and Hughes-Schrader. Although some trees did not provide support for the hypothesis of Schrader and Hughes-Schrader due to lack of resolution in the relevant part of the tree many trees did support this hypothesis, and in general it was the trees constructed using the most realistic assumptions which provided the greatest support.

Removal of the spinturnicids which are on the longest branch of the tree increased the resolution and bootstrap support in the remainder of the tree as well as the support for the hypothesis of Schrader and Hughes-Schrader in the neighbour joining and maximum parsimony analyses, but not in the maximum likelihood trees which already contained enough resolution to provide support for the hypothesis of Schrader and Hughes-Schrader. In summary, parsimony and neighbour joining methods supported the hypothesis once the amount of resolution in the tree was sufficient to be informative i.e. spinturnicids had been removed and the maximum likelihood method supported the hypothesis without the need for removal of the spinturnicids. It is not surprising that the maximum likelihood method copes better with long branches since that is one of the reasons why it was developed in the first place (see above). Although the different methods agree with respect to giving support to the hypothesis of Schrader and Hughes-Schrader, they nevertheless disagree about other parts of the tree, for example the position of the spinturnicids.

In conclusion, since there is no major disagreement between the three methods with respect to the hypothesis being tested, and since all indicate that the arrhenotokes arose from a pseudoarrhenotokous ancestor rather than directly from a diplodiploid zygogenetic one, this data set can unequivocally be said to support the hypothesis of Schrader and Hughes-Schrader. Furthermore it seems that there has been only a single origin of arrhenotoky within this group although this observation is more sensitive to sampling error than the test of the hypothesis of Schrader and Hughes-Schrader since addition of a single extra arrhenotoke to the tree is unlikely to lead to a tree which would contradict the hypothesis of Schrader and Hughes-Schrader (to do so it would have to attach to the tree at the base of the pseudoarrhenotokes) whereas it is more likely to constitute an independent origin of arrhenotoky (which would happen if it attached to the tree anywhere within the pseudoarrhenotokes other than at the point where the arrhenotokes already in the tree attach). In other words the chances that we have overlooked a taxon

which represents a contradiction of the hypothesis of Schrader and Hughes-Schrader are much lower than the chances that we have overlooked a taxon which represents an independent origin of arrhenotoky. (This assumes that although taxonomic sampling is sparse it is more or less evenly distributed across the group.)

Chapter Six

Combined Phylogenetic Analysis of 28S ribosomal DNA and Morphological Data Sets in the Dermanyssina (Acari: Mesostigmata)

SUMMARY

A morphological data set for the Dermanyssina (Strong, 1995) is analysed. This is in conflict with the molecular data set and does not support the hypothesis of Schrader and Hughes-Schrader. Combining the data in various ways (consensus of separately derived trees, analysis of total data with various weighting schemes) does not resolve the issue. Morphological data are mapped onto the tree derived from the molecular data in order to discover which morphological characters are in greatest conflict with the molecular data.

CONSTRUCTION OF THE COMBINED DATA SET

Taxa

The morphological data set for the Dermanyssina (Strong, 1995) contains more taxa than the molecular data set and not all of those represented in the molecular data set are also represented in the morphological data set. For these reasons pairs of taxa had to be chosen from these two different sources to represent putative clades in the combined data set (table 6.1). The criteria for choosing these pairs was different for the Dermanyssoidea and other superfamilies and were as follows:

Dermanyssoid Taxa

Representatives of dermanyssoid taxa were chosen from the morphological data set that were the closest to the taxa in the molecular data set in a tree derived from a second data set in Strong (1995) of morphological characters of the Laelapidae plus other dermanyssoid outgroups.

Non-Dermanyssoid Taxa

Representatives of non-dermanyssoid taxa were chosen from the morphological data set to be as close to the taxa in the molecular data set as possible (e.g. same species, same genus, same subfamily) according to the traditional taxonomy of the group.

Taxa in the molecular data set for which pairs could not be found (*Amblyseius cucumeris*, *Phytoseiulus persimilis*, *Spinturnix myoti*, *S. plecotinus* and *Hemipteroseius wormersleyi*)

Table 6.1: Taxa Represented in the Combined Data Set

Clade	Morphological Representative	Molecular Representative
Dermanyssoid Taxa		
Dermanyssus gallinae	*Dermanyssus gallinae*	*Dermanyssus gallinae*
Hypoaspis	*Hypoaspis blattae*	*Hypoaspis rosei*
Macrocheles	*Macrocheles muscadomestica*	*Macrocheles glaber*
Stratiolaelaps miles	*Stratiolaelaps miles*	*Stratiolaelaps miles*
Varroa + Haemogamasus	*Haemogamasus pontiger*	*Varroa jacobsoni*
Non-Dermanyssoid Taxa		
PHYTOSEIIDAE	*Typhlodromus pyri*	*Typhlodromus pyri*
VEIGAIIDAE	*Veigaia nemorensis*	*Veigaia nemorensis*
PARASITINAE	*Parasitus coleoptratorum*	*Cornigamasus lunaris*
PERGAMASINAE	*Pergamasus runcatellus*	*Pergamasus septentrionalis*

in the morphological data set were excluded from the combined analysis, but since the combined data set still contained zygogenetic, arrhenotokous and pseudoarrhenotokous members it could still be used to test the hypothesis of Schrader and Hughes-Schrader.

Characters

152 morphological characters were taken from Strong (1995) and added to the 755 molecular characters from chapter 5. This gave a combined data set of 889 characters. The morphological data set, molecular data set and combined morphological and molecular data sets used in this can be found in appendix 6.1.

PHYLOGENETIC ANALYSIS OF THE COMBINED DATA SET

Separate branch and bound parsimony analyses of the morphological and molecular data sets give conflicting results (figure 6.1).

Unfortunately, since the morphological data set contains only a single pseudoarrhenotoke it will be impossible to use this data set for a strict test of the hypothesis of Schrader and Hughes-Schrader. Although the molecular data set cannot be said to support the hypothesis of Schrader and Hughes-Schrader it does have the pseudoarrhenotoke as the sister taxon of a clade containing all of the arrhenotokes, the position which the hypothesis of Schrader and Hughes-Schrader would predict for it. This data set is therefore certainly not in conflict with the hypothesis of Schrader and Hughes-Schrader. It remains possible, however, that the ancestor of the clade consisting of the arrhenotokes

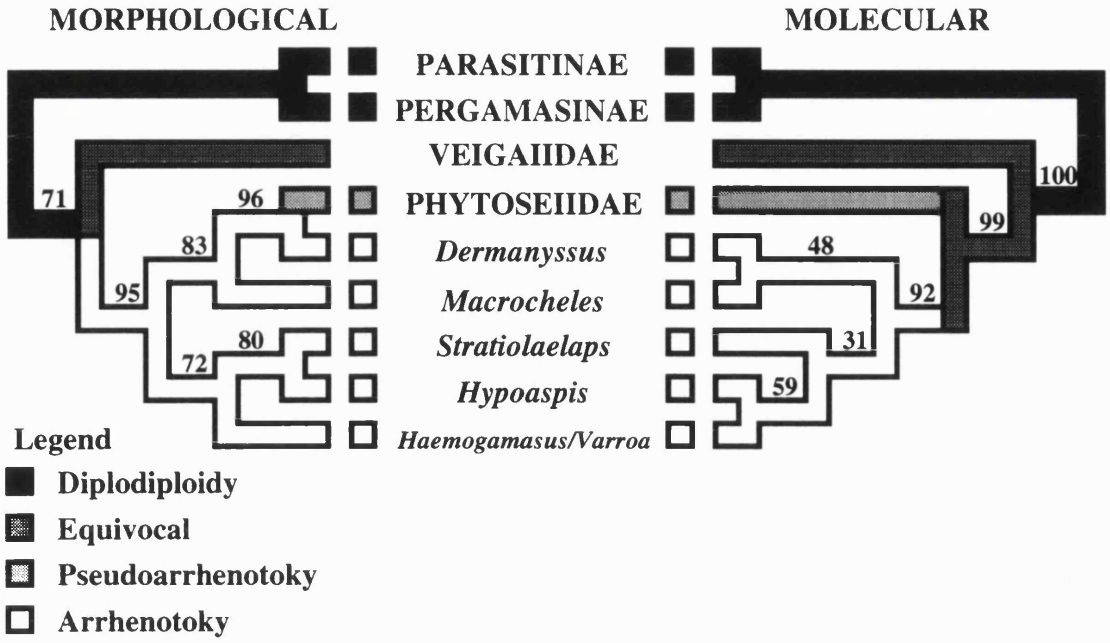

Figure 6.1: Maximum Parsimony Trees for the Morphological and Molecular Data Sets.

Figures above nodes indicate bootstrap support based on 100 replicates.

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plus the pseudoarrhenotoke is an arrhenotoke rather than a pseudoarrhenotoke which is the prediction of the hypothesis of Schrader and Hughes-Schrader. Only addition of another pseudoarrhenotoke to the tree can resolve this issue. In contrast, however, the morphological data set can be said unequivocally to conflict with the hypothesis of Schrader and Hughes-Schrader since in this tree the pseudoarrhenotoke arises from an arrhenotokous ancestor.

The two data sets also differ slightly in terms of the relationships within the arrhenotokous clade. The morphological data indicate that the sister group of the *Hypoaspis* is *Stratiolaelaps* (bootstrap support 80%). This is unsurprising since the *Stratiolaelaps miles* has previously been considered to belong to the genus *Hypoaspis*. On the other hand the molecular data group *Stratiolaelaps* with the putative clade representing *Haemogamasus* and *Varroa* (bootstrap support 59%). This may be a consequence of a false assumption that *Haemogamasus* and *Varroa* constitute a genuine clade.

A strict consensus of these two trees (figure 6.2) does not resolve the issue of whether these data support the hypothesis of Schrader and Hughes-Schrader since it does not resolve the nodes crucial for such a test.

Figure 6.2: The Strict Consensus of the Morphological and Molecular Trees

Parsimony analysis of the total evidence data set (i.e. the morphological and molecular data combined) recovered the same tree as the morphological data alone, even when the characters were weighted according to the number of informative sites in the data sets from which they came (i.e. because the morphological data set contained 82 informative sites whereas the molecular data set contained 56, weighting the molecular characters by 1.464 relative to the morphological characters meant that the two data sets made equal contributions to the total evidence data set).

Perhaps unsurprisingly the bootstrap proportions of those nodes found in both separate trees were generally higher in the total evidence trees than in the separate trees whereas the bootstrap proportions of those nodes found only in morphological tree were lower in the total evidence tree than they were in the morphological tree. These observations are consistent with the interpretation that the two data sets contain conflicting signals but that the signal in the molecular data is weaker than that in the morphological data set and indeed is *too* weak to prevent the signal from the morphological data from dominating in the total data set i.e. the molecular data fit the morphological hypothesis better than the morphological data fit the molecular hypothesis[116]. The nodes supported by both data sets (which tends to be towards the base of the tree) are not sufficient to resolve the question of whether the data support the hypothesis of Schrader and Hughes-Schrader. The morphological data contradicts the hypothesis of Schrader and Hughes-Schrader whereas the molecular data, although they do not support this hypothesis are at least entirely consistent with it. Since the morphological data set appears to have the stronger signal this might indicate that the morphological and molecular evidence taken as a whole

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[116] See Lee *et al.* (1997) for another example where this is the case.

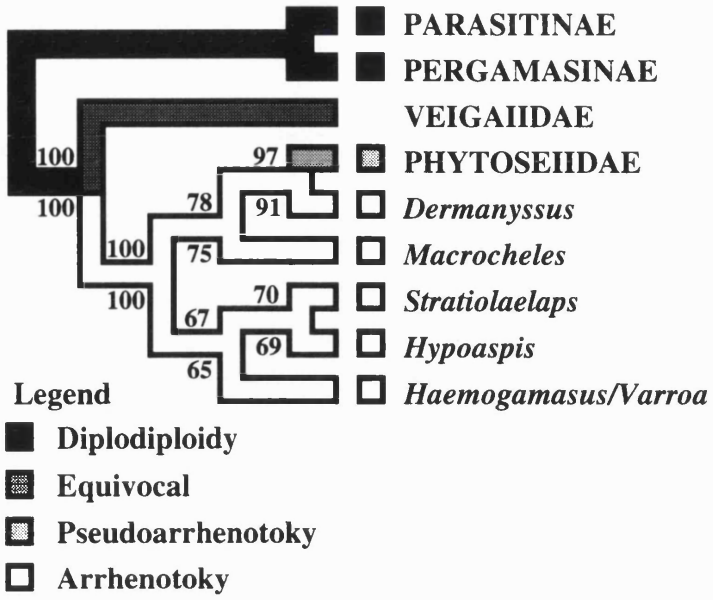

Figure 6.3: Maximum Parsimony Tree for the Total Data Set.

Figures above the nodes represent bootstrap support based on 100 replicates with all characters are weighted equally. Figures below the nodes indicate bootstrap support based on 100 replicates with the characters were weighted according to the number of informative sites in the data sets from which they came (see text).

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does not support the hypothesis of Schrader and Hughes-Schrader, however, before this conclusion is accepted it would be wise to examine the morphological data set more closely for evidence of internal conflict within this data set.

Internal conflict could be due to convergent evolution. Particular suites of characters which are particularly prone to convergent evolution may be dominating the data set and masking the signal from other characters which more genuinely reflect common ancestry. Removal of these unreliable characters may reveal an underlying congruence between the more reliable morphological characters and the molecular data set. Such an analysis can be achieved by breaking the morphological data set down into subsets and looking for evidence of conflict between these subsets. This method alone, however, will not tell us which of the conflicting subsets should be accepted and which rejected other than by appealing to the relative strengths of their phylogenetic signals (which we already know), but this may reflect poor choice of characters resulting in a data set dominated by convergent evolution rather than genuine phylogenetic signal. By constraining the tree topology to that given by the molecular data and then mapping the putative state changes of the morphological characters onto this tree we can identify which characters would exhibit the greatest degree of convergent evolution (homoplasy) if the molecular tree were correct. If we have *a priori* reasons for considering these characters particularly

likely to be prone to convergent evolution then we have a justification for removing them from the analysis.

In other words we need to ask three questions:

- What types of morphological characters are particularly prone to convergent evolution?
- Are these the characters which are in conflict with the molecular data?
- What is the effect of removing these characters from the analysis?

To answer the first question we could examine the amount of convergence each character exhibits in the morphological tree, however, this assumes that the majority of the data reflect genuine phylogeny whilst only a minority are confounded by the effects of convergent evolution. This is the very assumption we are attempting to test. Any estimates of the degree to which a character is prone to convergent evolution must come from evidence *independent* of our phylogeny if we are to avoid circularity in our reasoning.

Independent Evidence for Evaluation of Morphological Characters

What kinds of independent evidence are available for the evaluation of morphological characters for phylogenetic inference? Possibilities fall into two main types: empirical and statistical

Empirical Evidence

Certain types of character may be consistently poor at resolving genuine phylogenetic relationships for example characters which represent adaptations to certain environments will reflect shared ecology rather than shared ancestry.

Statistical Evidence

The differences in phylogenies proposed by different authors for the same taxa are often the result of subjective differences in which of several contradictory characters are assumed most likely to have uniquely derived (**convex**) character states. These putative convex characters are then used to determine the tree topology on which non-convex characters are assumed to have evolved in parallel (i.e. homoplastically). **Convexity compatibility assessment** (Camacho, Bello and Estabrook, 1997; Meacham and Estabrook, 1985) provides an objective statistical method for making such an assessment. The ideal is to exclude all non-convex characters, i.e. those which have the property that all phyletic line segments linking taxa with the same character states on the true tree also have this state. However, since we do not know the true tree we cannot do this. We can, however, identify pairs of characters which cannot possibly be convex on the same tree. Such characters are said to be **incompatible** (Estabrook, 1983; Estabrook and Landrum,

1975; Estabrook and McMorris, 1977). Convexity compatibility assessment chooses only those characters which are compatible with more other observed characters than 90% (or some other arbitrary cut-off value) of their randomly generated counterparts. Compatibility is assessed by the method of Estabrook (1993) as implemented in the program POTENT. Random characters are generated by the method of Meacham (1981) as implemented in the program CPSEQ (Meacham, 1994) in which taxa are randomly reassigned to states (Meacham, 1981). Maximum parsimony also chooses between different mutually incompatible sets of characters but differs from convexity compatibility assessment since it always chooses the largest set of compatible characters (or weights if characters are weighted unequally) represented in the data. Convexity compatibility assessment removes characters just as likely to be compatible with random characters as with other real ones. Such characters will therefore be of little phylogenetic value since a phylogenetic signal is exactly the same as a hypothesis of compatibility. Convexity compatibility therefore represents a genuine statistical test since it compares observations with expectations under a random model, rather then comparing different observations with each other.

MORPHOLOGICAL CHARACTER ANALYSIS

In order to identify which morphological characters are in greatest conflict with the morphological data set, the morphological characters are mapped onto the tree derived from the molecular data. Characters in conflict will have more than the minimum number of state changes possible on this tree (i.e. 1 - the number of states for that character). The degree to which a morphological character conflicts with the molecular data can therefore be expressed as m_i/s_i where m_i = the minimum number of character state changes and s_i = the observed number of character state changes. This is quantity is the consistency index (CI). Characters not in conflict will have a CI = 1, invariant characters by convention are given a CI = 0. Characters in conflict will have a CI between 0 and 1 and the lower the CI the greater the degree of conflict.

Strong (1995) divided the morphological characters into 15 groups but attempted no analysis of the relative merit of these different types of character for making valid phylogenetic inferences. Table 6.2 shows these morphological character types ranked in order of mean consistency index when mapped onto the molecular tree. Groups towards the top of this table are in greater conflict with the molecular data than those lower down. If the molecular tree were a better estimate of genuine phylogenetic relationships than the morphological tree then characters at the bottom of table 6.2 would represent 'good' markers of phylogeny whereas those towards the top would represent 'bad' ones.

But are the differences in consistency indices of different character types statistically significant? Consistency indices are not normally distributed and therefore either the data must be transformed or a non-parametric test must be used to ask whether different

Table 6.2: Morphological Character Types Ranked in Order of Mean Consistency Index

Morphological Character Type	Mean CI
DORSAL SHIELD	0.39
GNATHOSOMA	0.41
PERITREMES AND PERITREMATAL SHIELDS	0.42
EPISTOME (Tectum Capituli)	0.45
DORSAL SHIELD SETAE	0.47
STERNAL SHIELD	0.54
LEGS AND LEG CHAETOTAXY	0.55
EPIGYNIAL SHIELD (Female Genital Shield)	0.57
FEMALE CHELICERAE	0.57
PEDIPALPS	0.64
ANAL SHIELD	0.64
FEMALE REPRODUCTIVE CHARACTERS	0.67
OPISTHOGASTRIC REGION OF VENTRAL SHIELD	0.67
MALE CHARACTERS	0.75
MALE CHELICERAE	0.83

character types have significantly different consistency indices (i.e. whether they differ significantly in the degree to which they conflict with the molecular data). In this case the Kruskal-Wallis test is the most appropriate (Hollander and Wolfe, 1973; Kruskal, 1952; Kruskal and Wallis, 1952; Siegel and Castellan, 1988; Sokal and Rohlf, 1995). This test can be applied to specific hypotheses by partitioning the data in different ways e.g. reproductive vs. non-reproductive or dorsal vs. ventral. If it is indeed true that the characters which we expect to exhibit the greatest degree of convergence are those which are in greatest conflict with the molecular data set then this may indicate that the molecular data set is the better indicator of phylogeny and that the morphological data set as a whole is a poor indicator of phylogeny and contains large amounts of homoplasy.

Uncorrected H = (0.001763150162 ((the sum of rank sums over all a)^2/n_i)) - 249, where a = the number of categories

D = 1-((the sum of T_j over all m)/((N_i-1)(N_i)(N_i+1))), where $T_j = t_j^3-t_j = (t_j-1)(t_j)(t_j+1)$, m = the number of tied groups, N_i = the sum of n_i over all a and t_j = the number of variates tied in the jth tied group

Corrected H = Uncorrected H/D

For a>3, corrected H has a chi squared distribution with a-1 degrees of freedom.

uncorrected H	21.39
D	0.829
corrected H	25.80
df	14
P	0.05>P>0.025
Significance	significant

i.e. there is a significant difference between the different groups in the degree to which they conflict with the molecular data. This test does not indicate which group or groups are significantly different from the rest. There are three possible approaches to discovering this:

- Repeat the Kruskal-Wallis test for pruned subsets of the groups
- Divide the groups into subsets by graphical methods
- Construct maximum parsimony trees with pruned subsets of groups

The approach taken here is construction of maximum parsimony trees with pruned subsets of groups. Groups of morphological characters were successively removed from the total data set in the order in which they appear in table 6.2. i.e. in increasing order of consistency index or decreasing order of the degree to which they conflict with the molecular data. The tree topology did not change from that given by the total data (and the morphological data alone) until the first seven groups of morphological characters in table 6.2 had been removed. This means that these seven groups constitute those groups which are in some way in conflict with the total data set. These groups are:

Dorsal shield
Gnathosoma
Peritremes and Peritrematal Shields
Epistome (tectum capituli)
Dorsal Shield Setae
Sternal Shield
Legs and Leg Chaetotaxy

The maximum parsimony tree given by the total data set with these characters removed was the same as that given by the molecular data alone except for the relationships within the arrhenotokous clade (figure 6.4). This difference between the total data with these characters removed and the molecular data has no effect on the relationships *between* the genetic systems and therefore will not effect any test of Schrader and Hughes-Schrader and in any case, as noted above, this difference may simply be an artifact a false assumption that *Haemogamasus* and *Varroa* constitute a genuine clade.

A quick glance at table 6.2 shows that most of the reproductive and sexually dimorphic characters are towards the bottom of this table i.e. do not conflict strongly with the

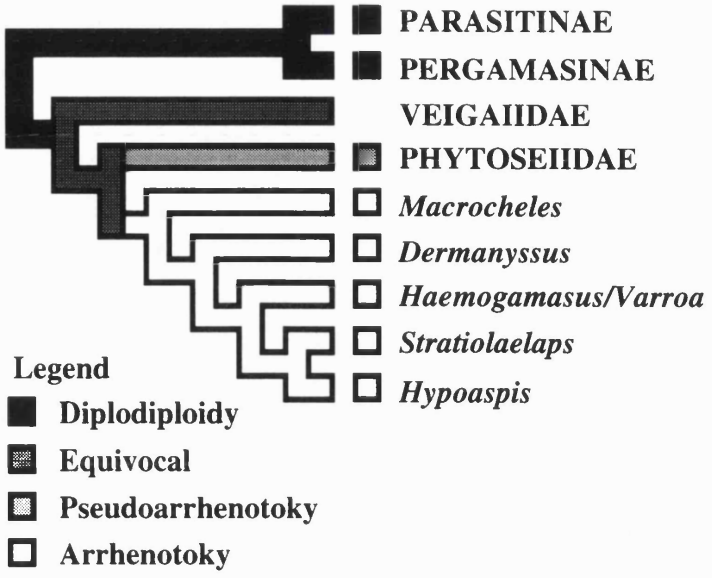

Figure 6.4: Maximum Parsimony Tree for Pruned Total Data Set
(See text for details)

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molecular data. (Notice for instance that male chelicerae which have a role in reproduction in these mites (see appendix 5.6) exhibit much less character conflict than female chelicerae which do not have a reproductive role.) None of the seven groups of conflicting characters are reproductive whereas four of the remaining eight groups i.e. 50% of the groups not in conflict with the molecular data consist of reproductive characters. Reproductive characters may be better indicators of phylogeny due to lower degrees of convergence than non-reproductive characters. Reproductive characters are often favoured by morphological systematists possibly because changes in reproductive characters may lead to reproductive isolation and ultimately speciation whereas non-reproductive characters are more likely to reflect the operation of natural selection which is more strongly correlated with environment than with phylogeny i.e. reproductive characters in common between two species are likely to represent common ancestry whereas shared non-reproductive characters may reflect a shared environment.

Although a Kruskal-Wallis test of reproductive versus non-reproductive characters is not significant[117], this is a conservative test. Removal of non-reproductive characters from the total data set gives the same relationship between the genetic systems as the molecular data alone, although the relationships within the arrhenotokes are different again from

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[117] Reproductive character groups were considered to be epigynial shield, female reproductive characters, male characters and male chelicerae. For reproductive characters rank sum = 787, n_i = 17, (rank sum)$^2/n_i$ = 36433. For non-reproductive characters rank sum = 2616, n_i = 65, (rank sum)$^2/n_i$ = 105284. Total = 141717. Uncorrected H = 0.869, Corrected H = 1.048 (D = 0.829, a = 2). For a = 2, H is not χ^2 distributed so an exact statistic for the Kruskal-Wallis test must be used.

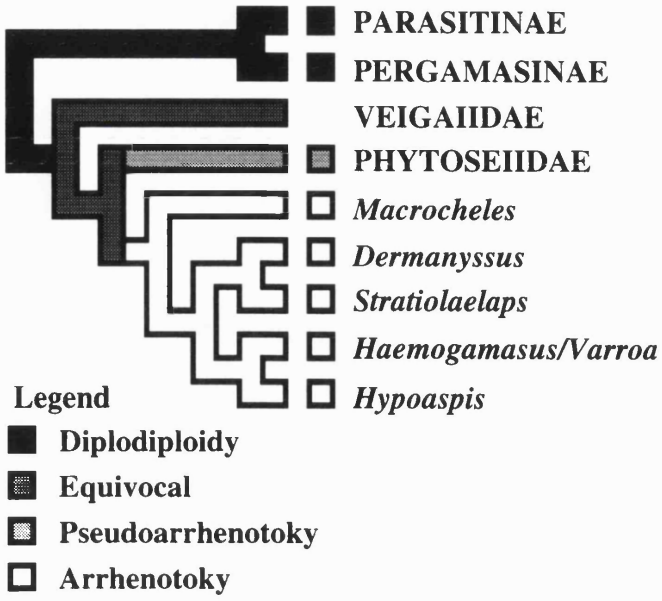

Figure 6.5: Maximum Parsimony Tree for Molecular Data Plus the Reproductive Morphological Characters

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both the molecular tree and from the morphological tree which is the same as the total evidence tree with all morphological characters included (figure 6.5). The reproductive characters alone do not contain enough resolution to test the hypotheses of Schrader and Hughes-Schrader since a strict consensus of 11 maximally parsimonious trees contains only a single internal branch which separates the ingroup from the outgroup.

The fact that the morphological characters which agree best with the molecular characters are, to some extent at least, those which we might expect, for independent reasons, to best reflect phylogeny (i.e. reproductive characters) may mean that the molecular characters and the reproductive characters do in fact contain genuine phylogenetic information which is swamped by noise from the other, non-reproductive characters in the total data set. This may be reflected in the fact that the total data set with non-reproductive morphological characters removed has a more left skewed tree length distribution (g1 = -1.269) than the total data set with the non-reproductive included (g1 = -1.050) suggesting that the data set with the non-reproductive characters removed contains greater hierarchical structure. (G1 statistics are based on 100000 random trees. See chapter 5 for further explanation of g1 statistics as measures of hierarchical structure.)

Chapter Seven

Conclusions: A Solution to the Problem of Male Haploidy and Ideas for Future Research

"The .... foregoing considerations are concerned with the mode of origin of haploidy. It will be seen that the central point of why such haploid individuals should always be of the male sex still remains. It is, however, more than probable that with a complete elucidation of the origin of haploidy this final question will be automatically answered. If our conclusions still fall short of that desideratum it must be realised that the defect lies in the fact that our analysis of the origin of haploidy is still incomplete. However, in so far as these investigations offer an approach to the central objective, they may have accomplished something towards its final solution."

Franz Schrader and Sally Hughes-Schrader, 1931

COMBINATORIALIST APPROACH TO HAPLODIPLOIDY AND A SOLUTION TO THE PROBLEM OF MALE HAPLOIDY

Clearly, judging from the quote cited above, Schrader and Hughes-Schrader did not think that their hypothesis alone would be able to solve the problem of why it is always males which are the haploid sex, however, if we generalise their hypothesis to all uniparental genetic systems and then examine its consequences for each of the four basic systems listed in chapter 1 then it can be shown that the generalised hypothesis of Schrader and Hughes-Schrader predicts that at least among postfertilisation systems those in which in which males are haploid are expected to be more common. This method of examining the consequences of all possible variations of some system to discover why in fact only some subset of all of these actually occurs, is known as the combinatorialist approach:

"In the simplest analysis one may observe that certain forms of a system are present and others are absent. This approach represents the combinatorialist perspective, i. e., the categorization of known systems among those which are theoretically possible. The value of the combinatorialist approach is simply that it enables the investigator to consider the possible and perhaps probable existence of as yet undiscovered variety. This investigative process in turn may lead to the recognition of new variety or to the realization that some forms are invariably absent....

....The presentation of the variety of mechanisms known, coupled with the variety possible could well stand on its own as a self-contained discipline in evolutionary biology. That is, one would be justified in looking no deeper. However, this appreciation for the variety of mechanisms is an almost essential prerequisite for the second objective, which is to understand how the different mechanisms evolve."

James J. Bull, 1983

The combinatorialist method has been widely used in the mathematical and physical sciences (Eddington, 1929). Its use in evolutionary biology is perhaps more recent but goes back at least as far as the famous quote by R. A. Fisher, himself a mathematician, from the preface to his 1930 book *The Genetical Theory of Natural Selection*:

> *No practical biologist interested in sexual reproduction would be lead to work out the detailed consequences experienced by organisms having three or more sexes; yet what else is he to do if he wishes to understand why the sexes are, in fact, always two?*
>
> *R. A. Fisher, 1930*

The Special Hypothesis of Schrader and Hughes-Schrader

As has been discussed in great detail in previous chapters the hypothesis of Schrader and Hughes-Schrader suggests that arrhenotoky arises not directly from ancestral diplodiploid zygogenesis but instead from a pseudoarrhenotokous intermediate. This I will refer to as the **special** hypothesis of Schrader and Hughes-Schrader.

The General Hypothesis of Schrader and Hughes-Schrader

Since arrhenotoky is a prefertilisation uniparental genetic system and pseudoarrhenotoky is a postfertilisation system (see chapter 1) the special hypothesis of Schrader and Hughes-Schrader can be generalised to cover all uniparental genetic systems i.e. the **general** hypothesis of Schrader and Hughes-Schrader states that all prefertilisation uniparental genetic systems arise not directly from ancestral diplodiploid zygogenesis but instead from a corresponding postfertilisation intermediate. It is important to note at this point that Schrader and Hughes-Schrader never suggested this generalised formulation of their hypothesis themselves and there is no evidence that any prefertilisation genetic system other than arrhenotoky arose via a postfertilisation intermediate but this is to be expected since such systems are so rare. Nevertheless this generalised formulation of the hypothesis is highly speculative and is only meant to be tool in a thought experiment. It may be instructive to proceed as if the general hypothesis of Schrader and Hughes-Schrader were true and work out the consequences of it being true. If these predicted consequences correspond well to what we observe in the real world then this may indicate that the generalised hypothesis may have some validity but this must be confirmed with a great deal more empirical and experimental research before it can be accepted more fully.

The essential point of the generalised hypothesis of Schrader and Hughes-Schrader is that since prefertilisation systems must arise from postfertilisation systems the existence of such postfertilisation systems acts as a constraint on the evolution of prefertilisation systems. The absence of a particular prefertilisation system may be due to constraints on that prefertilisation system itself or on constraints on the postfertilisation system which is a necessary intermediate in its evolution from ancestral diplodiploid zygogenesis.

There are four basic uniparental systems which are described in detail in chapter 1. These are paternal sons, paternal daughters, maternal sons and maternal daughters. Each of these can be either a postfertilisation genetic system or a prefertilisation genetic system. the general hypothesis of Schrader and Hughes-Schrader predicts that prefertilisation paternal sons will evolve from postfertilisation paternal sons, prefertilisation paternal daughters from postfertilisation paternal daughters, prefertilisation maternal sons form postfertilisation maternal sons and prefertilisation maternal daughters from postfertilisation maternal daughters.

Prefertilisation Systems

Both prefertilisation systems in which males are the uniparental parent (see chapter 1) (i.e. prefertilisation paternal sons and prefertilisation paternal daughters) are unknown, whereas both of those in which the female is the uniparental parent (i.e. prefertilisation maternal sons (arrhenotoky) and prefertilisation maternal daughters (thelytoky)) are common. This distribution of prefertilisation systems can easily be explained in terms of constraints on androgenesis. Since in a prefertilisation genetic system the uniparental parent must produce offspring asexually prefertilisation systems in which the female is the uniparental parent (which are common) are characterised by parthenogenesis. Systems in which the male is the biparental parent are unknown would be characterised by androgenesis, however, there are strong constraints on androgenesis (see chapter 1) and for this reason there are strong constraints on any prefertilisation genetic system in which males are the uniparental parent. This information is summarised in table 7.1.

Table 7.1: Distribution of Prefertilisation Genetic Systems

		UNIPARENTAL PARENT	
		Male	**Female**
UNIPARENTAL	**Male**	Unknown*	Common (e.g. arrhenotoky)
OFFSPRING	**Female**	Unknown*	Common (e.g. thelytoky)

* Due to constraints on androgenesis

This still does not solve the problem of male haploidy. We have excluded paternal systems on the grounds that they are subject to large constraints and observational bias but what about maternal systems in which females arise uniparentally and may therefore be haploid. Systems in which females arise uniparentally from their mothers do not require males and these will rapidly be lost since their production will involve costly investment of resources which could otherwise be used to produce females. For this reason all prefertilisation maternal daughter systems will evolve in the direction of

thelytoky. This means that the only prefertilisation systems which should exist should be arrhenotoky (in which males are haploid), thelytoky (in which females may be haploid or diploid) and a much lower number of systems such as prefertilisation maternal daughter systems which still retains males (there is one known example of this - see chapter 1) and pseudogamy which are on their way to evolving into thelytoky[118]. This is exactly the pattern of prefertilisation systems found in nature (see figure 7.1).

Postfertilisation Systems

Both postfertilisation systems in which males are the uniparental parent (i.e. postfertilisation paternal sons and postfertilisation paternal daughters) are unknown, whereas both of those in which the female is the uniparental parent (i.e. postfertilisation maternal sons (pseudoarrhenotoky) and postfertilisation maternal daughters (pseudogamy)) are common. This pattern is the same as that in the prefertilisation systems i.e. all uniparental genetic systems in which the uniparental parent is male are unknown whereas all of those in which the uniparental parent is female are common. At first sight this pattern is more puzzling in the postfertilisation systems since there are no constraints on androgenesis in a postfertilisation system, however, there are two possible explanations for the apparent lack of postfertilisation uniparental genetic systems in which the uniparental parents are male: maternally inherited cytoplasmic effects and observational bias (see figure 7.1). This information is summarised in table 7.2.

Table 7.2: Distribution of Postfertilisation Genetic Systems

		UNIPARENTAL PARENT	
		Male	**Female**
UNIPARENTAL	**Male**	Unknown*	Common (e.g. pseudoarrhenotoky)
OFFSPRING	**Female**	Unknown*	Common (e.g. pseudogamy)

* Due to cytoplasmic effects or observational bias

Cytoplasmic Effects

There may be a constraint on the evolution of postfertilisation systems in which the uniparental parent is male. Since according to the general hypothesis of Schrader and Hughes-Schrader the existence of a postfertilisation system is a prerequisite for the evolution of a prefertilisation system real reason for a lack of prefertilisation systems in which the male is the uniparental parent may be due to this constraint rather than to

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[118] This is a dynamic equilibrium. The number of these intermediate systems will be a function of the rate at which they arise, and the rate at which they go extinct or evolve into other systems.

constraints on androgenesis. Constraints on postfertilisation uniparental genetic systems in which males are the uniparental parent may be due to maternally inherited cytoplasmic factors which will tend to prevent the elimination of the maternal genome. Since males contribute no such factors to the offspring they are less able to prevent the elimination of the genome which they contribute to their offspring and this leads to the evolution of postfertilisation genetic systems in which the female is the uniparental parent and the subsequent evolution of corresponding prefertilisation systems.

Observational Bias

As has been noted in earlier chapters, there is considerable observational bias against the discovery of postfertilisation systems which do not have relatives with prefertilisation systems. According to this view all postfertilisation systems have arisen and exist in more or less even proportions but only those which are related to prefertilisation systems, which have drawn the attention of researchers to these groups, have been discovered. Since the only prefertilisation systems which exist are ones in which females are the uniparental parents it follows that the only postfertilisation systems which have been discovered are also those in which females are the uniparental parents. There may be postfertilisation systems in which males are uniparental parents awaiting discovery but our attention has not been drawn to them. This assumes that postfertilisation systems are less likely to be serendipitously discovered, which is probably true since a prefertilisation systems can be inferred simply from an observation production of offspring by virgins whereas postfertilisation systems require complicated cytogenetic techniques.

IDEAS FOR FUTURE RESEARCH

Extending the Test of the Special Hypothesis of Schrader and Hughes-Schrader in the Dermanyssina

As mentioned in chapter 5 there are a number of ways in which this project could be extended. Simply adding more taxa to the tree would be a valuable exercise. In general addition of any taxa to the analysis would be an improvement but there are a number of specific taxa which it would be of particular interest to include.

Ingroup Sampling

Ingroup taxa which it would be desirable to add to the analysis given in chapter 5 include the genus *Gamasellodes*, the superfamily Rhodacaroidea, the families Ameroseiidae, Ascidae and Antennoseiidae and the species *Macrocheles mycotrupetes*.

Gamasellodes

It would be of considerable interest to add a representative of the genus *Gamasellodes* which is the only arrhenotokous member of an otherwise exclusively

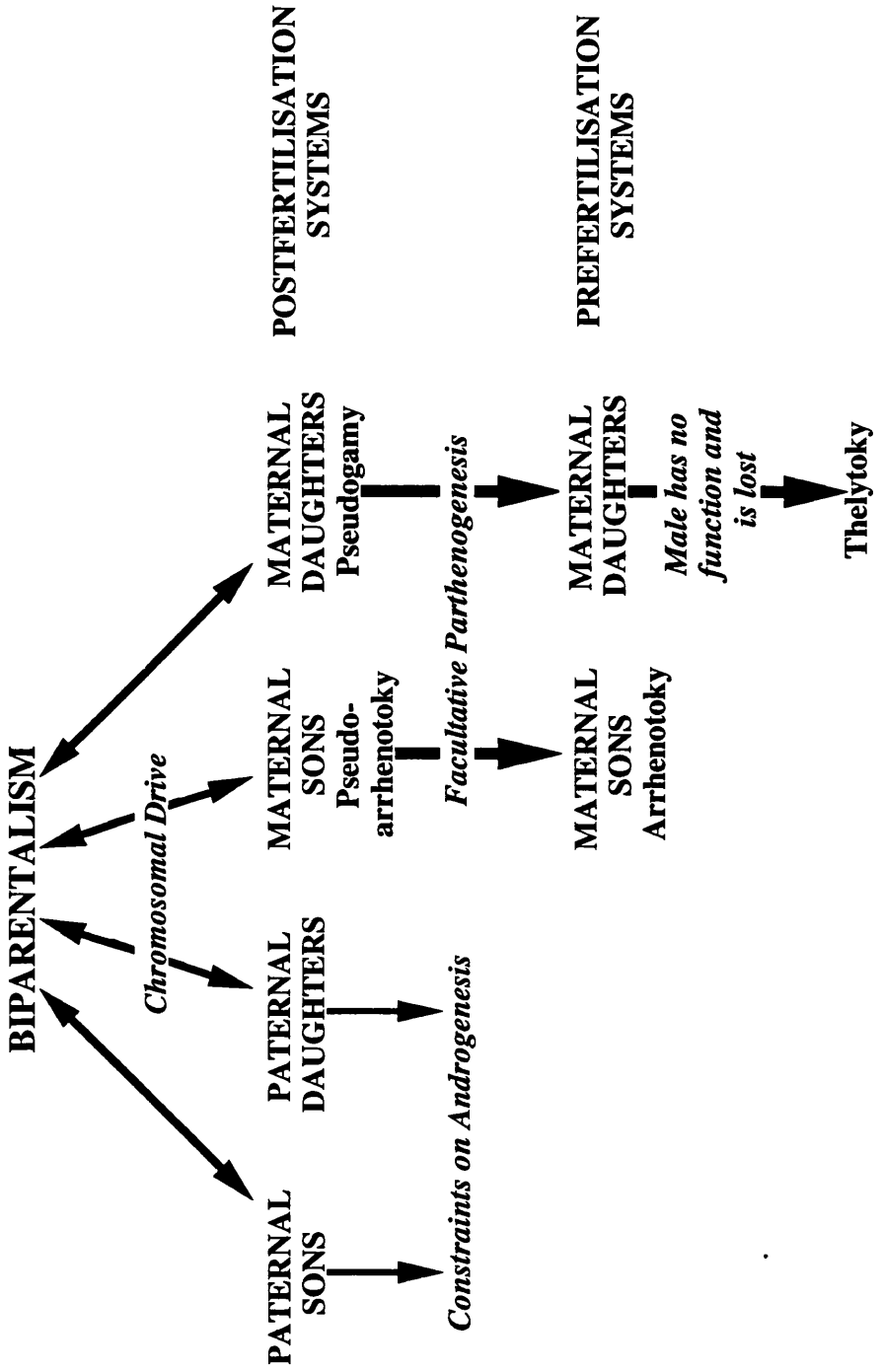

Figure 7.1: A scheme for the evolution of uniparental genetic systems.

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pseudoarrhenotokous superfamily, the Ascoidea. *Gamasellodes* may therefore represent an independent origin of arrhenotoky within the Ascoidea or it may be at the base of an arrhenotokous clade represented by the Dermanyssoidea and Eviphidoidea which seems to arise from within the pseudoarrhenotokous Ascoidea (see chapter 3).

Rhodacaroidea

David Walter (pers. comm. 1994) considers the superfamilies Rhodacaroidea and Ascoidea to be ill defined since the separation of Rhodacaroidea from Ascoidea depends on the relative degree of development of ventral shields and leg chaetotaxic reductions, characters he considers particularly susceptible to convergence, a conjecture which agrees with the conlusions of the morphological character analysis presented in chapter 6 (see table 6.2). For example he places the genus *Gamasellodes* in the Rhodacaroidea in which case the entire superfamily Ascoidea may be pseudoarrhenotokous and arrhenotoky may have evolved directly from diplodiploidy in the Rhodacaroidea. The position of this genus as well as the unsampled superfamily Rhodacaroidea may therefore be crucial to a test of the hypothesis of Schrader and Hughes-Schrader.

Families Ameroseiidae, Ascidae and Antennoseiidae

Walter (pers. comm. 1994) also removes the families Phytoseiidae, Podocinidae and Otopheidomenidae from the Ascoidea and elevates them to a new superfamily, the Phytoseiioidea, defined by the apomorphic character of the 'phytoseiioid type' of sperm access system (see appendix 7.1). This change in reproductive morphology may be related to a change in genetic system i.e. this may be the only pseudoarrhenotokous lineage with what is left in the Ascoidea *sensu* Walter being exclusively diplodiploid. Since the only members of the Ascoidea included in the phylogeny are pseudoarrhenotokes and belong to Walter's Phytoseiioidea it is not possible to use this data set to test this hypothesis until representatives of the non-phytoseiioid Ascoidea (i.e. Ameroseiidae, Ascidae and Antennoseiidae) are included.

Macrocheles mycotrupetes

Another taxon of interest not included in the present tree is *Macrocheles mycotrupetes*. This species which seems to be primitive within the otherwise arrhenotokous Macrochelidae on morphological grounds does not produce progeny without mating and is therefore unlikely to be arrhenotokous (see chapter 3). This suggests that a switch to arrhenotoky may have occurred within the Macrochelidae (either from pseudoarrhenotoky or directly from a diplodiploid zygogenetic ancestor depending on the precise genetic system of *Macrocheles mycotrupetes* which has yet to be discovered) which subsequently gave rise to the large radiation of arrhenotokes. This scheme would suggest a paraphyletic Macrochelidae at the base of the arrhenotokes. The taxonomic sampling presented in chapter 5 is insufficient to test this hypothesis, particularly in view of the fact that the *Macrocheles glaber* is not consistently found at the base of the arrhenotokes (see chapter 5).

Outgroup Sampling

As well as improving the sampling of ingroup taxa it may also be of interest to sample more outgroup taxa, particularly in view of some of the ideas of David Walter (pers. comm. 1994). He cites biogeographical evidence suggesting that contrary to the hypothesis of Johnston in Norton *et al.* (1993) on which the selection of Parasitina as an outgroup was based (figure 7.2a) the Dermanyssina are basal to a clade consisting of the cohorts Epicriina + Zerconina[119] + Arctacarina + Parasitina (figure 7.2b). This evidence is based on the relative lack of these cohorts and abundance of Dermanyssina (particularly Rhodacaroidea) in Australia suggesting that these cohorts constitute a monophyletic clade to which the Dermanyssina is a sister taxon rather than being the most derived group within it. This would make Parasitina an inappropriate outgroup and instead suggest as an outgroup Uropodina, Diarthrophallina[120], Microgyniina or Sejina.

Investigation of Unknown Genetic Systems

At first sight it might seem that the hypothesis of Schrader and Hughes-Schrader makes specific hypotheses about the genetic systems of some of the taxa included in the analysis in chapter 5 but for which the genetic systems are unknown, based on their position in the tree. If this were true then investigation of the genetic systems of these taxa would therefore provide a further test of this hypothesis. In particular it might be expected that the hypothesis of Schrader and Hughes Schrader would predict from the position of *Veigaia nemorensis* that it is not arrhenotokous. This however is not the case since *Veigaia nemorensis* could represent an independent origin of arrhenotoky from a pseudoarrhenotokous ancestor i.e. the trees in chapter 5 are entirely consistent with the possibility that the switch to pseudoarrhenotoky occurred early in the Dermanyssina i.e. prior to the diversification of the Veigaioidea and since the hypothesis of Schrader and Hughes-Schrader says nothing about the frequency of evolution of arrhenotoky merely that when it does arise it arises from a pseudoarrhenotokous ancestor rather than a diplodiploid zygogenetic one. The principle of parsimony would, of course, predict that *Veigaia nemorensis* is not arrhenotokous since this would postulate an extra step on the tree but it is not the principle of parsimony which we wish to test. Since the position of the spinturnicids is not stable on the tree an elucidation of the genetic systems of these two taxa would be even less useful. For these reasons the best approach to extending this project would be to add more taxa to the tree rather than to attempt an elucidation of those taxa for which the genetic systems are unknown.

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[119] The monofamilial cohorts Epicriina (*sensu* Evans, 1992) and Zerconina were previously grouped together in the cohort Epicriina (*sensu* Johnston, 1982).

[120] The cohort Diarthrophallina consists of a single family (Diarthrophallidae) considered by Norton *et al.* (1993) to be a derived family of the cohort Uropodina but by Evans (1992) to be a separate cohort.

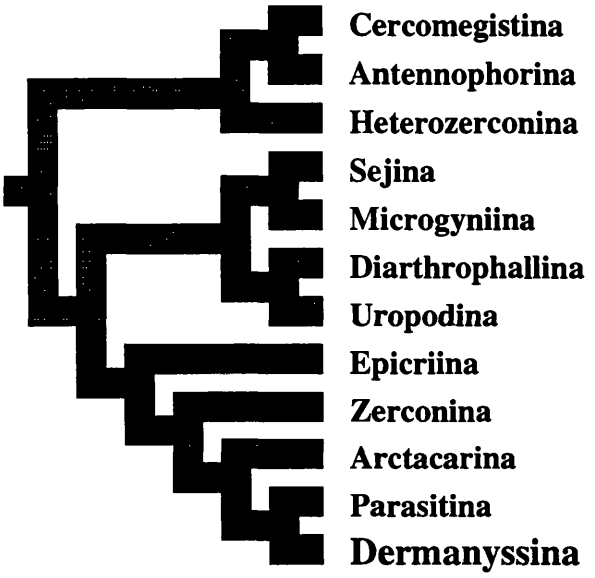

Figure 7.2a: The phylogeny of the cohorts of the Mesostigmata according to Johnston in Norton *et al.* (1993) but with Diarthrophallina considered as a separate cohort

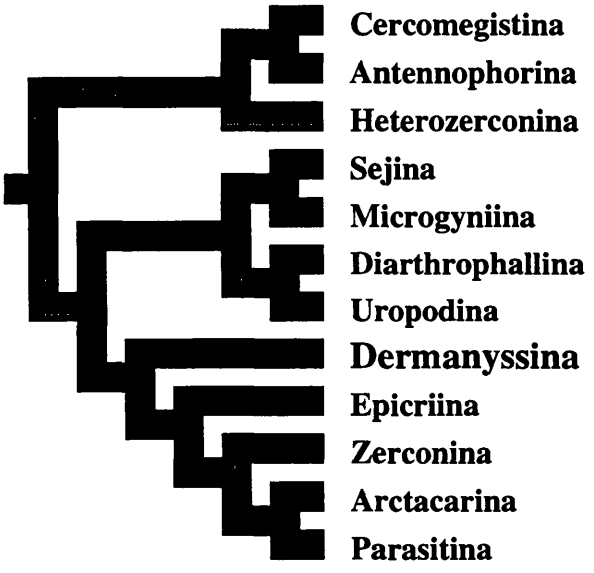

Figure 7.2b: The phylogeny of the cohorts of the Mesostigmata according to Johnston in Norton *et al.* (1993) but with Diarthrophallina considered as a separate cohort and the position of the Dermanyssina altered according to the hypothesis of Walter (pers. comm. 1994)

Testing the Special Hypothesis of Schrader and Hughes-Schrader in Other Groups of Mites

As discussed in chapter 3 pseudoarrhenotoky has never been proven in any mites outside the Dermanyssina. Any test of the hypothesis of Schrader and Hughes-Schrader would require either living pseudoarrhenotokes to be present in a group which also contains

arrhenotokes or for independent evidence that some now extinct ancestor of a group of arrhenotokes was pseudoarrhenotokous (although it is hard to imagine what would constitute such evidence.) The absence of living pseudoarrhenotokes in modern groups which also contain arrhenotokes cannot be taken as evidence against the hypothesis of Schrader and Hughes-Schrader as it was by Hartl and Brown (1970) (see chapter 3). This should be interpreted as absence of evidence rather than evidence of absence, particularly since there is good reason to think that pseudoarrhenotoky is likely to be less stable than arrhenotoky (see chapter 2). In other words the fact that not all groups which contain arrhenotokes also contain pseudoarrhenotokes is not surprising and is entirely consistent with the hypothesis of Schrader and Hughes-Schrader. On the other hand the fact that all groups which contain pseudoarrhenotokes also contain arrhenotokes is highly suggestive that the two systems are phylogenetically related and it is only in such groups that the hypothesis of Schrader and Hughes-Schrader can be tested. For this reason, while pseudoarrhenotokes are not found among any of the other haplodiploid groups of mites, at present these groups cannot be used to test this hypothesis. It is entirely possible however, that pseudoarrhenotokes will be discovered in the future in these groups and research on the evolution of haplodiploidy in these groups should be directed towards elucidating the precise nature of the genetic systems in those groups such as the Cunaxidae (Prostigmata), the Brachypylina (Sarcoptiformes) and the Histiostomatoidea (Astigmata) (see chapter 3) in which there is still much confusion. Should any of these investigations show conclusive evidence of the existence of pseudoarrhenotokes in these groups then this could provide the basis for further tests of the hypothesis of Schrader and Hughes-Schrader.

Testing the Special Hypothesis of Schrader and Hughes-Schrader in Other Taxa

For similar reasons the Hymenoptera, Thysanoptera and Oxyuroidea are unlikely to provide a great deal of information about the evolutionary origins of haplodiploidy. In fact they are probably less likely to do so than the acariform mites since it seems very unlikely that any as yet unrecognised pseudoarrhenotokous ancestor of these groups remains to be discovered. The phylogenetic relationships between the various genetic systems of the Coccoidea are well understood (see chapters 1 and 3). The situation is complicated with two apparently independent origins of arrhenotoky one of which retains an extant pseudoarrhenotokous ancestor and thus agrees with the hypothesis of Schrader and Hughes-Schrader and a second which has no pseudoarrhenotokous ancestor but which for reason outlined above cannot be said to contradict the hypothesis.

A third group in which pseudoarrhenotoky and arrhenotoky occur together is the Scolytid beetles and it would be of considerable interest to test the hypothesis of Schrader and Hughes-Schrader within this group. At present only a single species, the coffee berry borer (*Hypothenemus hampei*) has been identified as a pseudoarrhenotoke. It may be due to the economic importance of this pest species that it was ever investigated at all and a

great number of as yet unknown pseudoarrhenotokes which are not so economically important may have been overlooked. The precise nature of the relationship of this beetle to its arrhenotokous relatives would constitute another independent test of the hypothesis of Schrader and Hughes-Schrader, particularly in combination with a thorough investigation of the genetic systems of other members of this family. Any test of the origin of arrhenotoky in the primitive beetle *Micromalthus debilis* (which is so far from the Scolytidae in terms of evolutionary distance that it undoubtedly represents another independent origin of arrhenotoky) is highly unlikely since it is so different from any other beetle that the probability of finding a living pseudoarrhenotokous relative is extremely remote.

Testing the General Hypothesis of Schrader and Hughes-Schrader

A phylogenetic test of the general hypothesis of Schrader and Hughes-Schrader would require groups which contain both postfertilisation and prefertilisation forms of each of the basic uniparental genetic systems outlined in chapter 1. Since the only basic uniparental genetic system for which such groups exist is maternal sons at the present time no test of the general hypothesis of Schrader and Hughes-Schrader can be made.

Dating the Nodes in the Phylogeny

It would be of considerable interest to be able to put dates on some of the nodes in the phylogenies constructed in chapter 5. The data do not conform well to a molecular clock model, for example the two spinturnicids are on a very long branch indicating that the rate of evolution of 28S rDNA is considerably higher in these mites than elsewhere in the tree. Another problem is the lack of a good fossil record which is a requirement for such an analysis (Benton, 1996; Smith, 1994). Only two fossil mesostigmatid mites have been reported, both from the mid Tertiary. These are a Phytoseiid, *Seius bdelloides*, from Baltic amber (Koch and Berendt, 1854) and a Digamasellid, *Dendrolaelaps fossilis*, from the Chiapas amber of Mexico (Hirschmann, 1971). The situation can, however, only improve:

> *"Fossil mites are probably found routinely in palynological preparations but are unreported. With the growth of micropalaeontological techniques in the study of fossil arthropods it is likely that many more fossil mites will be identified."*
>
> *Paul A. Seldon, 1993*

Seldon's prediction is beginning to come true, for example Braun (1997) provides detailed pictures of a number of recently discovered, but as yet undescribed, fossil mites from the Devonian and Carboniferous found using new micropalaeontological techniques. It is hoped that as new fossil mites come to light an attempt may be made to put dates on the nodes in the phylogeny but the present state of the fossil record is insufficient to permit such an analysis.

Appendix 4.1

Protocols for the Methods of DNA Extraction Used in Chapter Four

DNA EXTRACTION METHOD #1

1 Add 2µl of L6 lysis buffer[121] to a sterile 1.5 ml Eppendorf tube containing a single mite.

2 Crush the mite with a pestle made by melting a 1ml pipette tip and moulding it into the bottom of a 1.5ml Eppendorf tube.

3 Mix.

---

[121] **L6 lysis buffer**

1 Heat 120g of Guanidinium thiocyanate in 100ml of 0.1M Tris HCl pH6.4 (see below) until dissolved.

2 Add 22ml 0f 0.2M EDTA pH8.0 (see below).

3 Add 2.6g of Triton X-100.

4 Mix by vortexing.

5 Store in the dark at room temperature.

0.1 M Tris HCl pH6.4

1 Add 1.211g of Tris to 100ml of water.

2 Adjust to pH6.4 with HCl.

3 Store at room temperature.

0.2M EDTA pH8.0

1 Add 8.8ml of 0.5M EDTA pH8.0 (see below) to 13.2ml of water.

2 Store at room temperature.

0.5M EDTA pH8.0

1 Add 186.1g of Disodium ethylenediaminotetra-acetate.2H2O to 800ml of water.

2 Stir vigorously with a magnetic stirrer.

3 Adjust the pH to 8.0 with Sodium Hydroxide (about 20g of NaOH pellets). (The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to about 8.0 by the addition of NaOH (Sambrook, Fritsch and Maniatis, 1989).

4 Dispense into aliquots.

5 Sterilise by autoclaving.

6 Store at room temperature.

4 Incubate at 55°C for 10 minutes.
5 Add 18µl of Sodium Iodide buffer[122].
6 Add 2µl of Glassmilk[123].
7 Incubate at room temperature for 5 minutes vortexing after 2 and 4 minutes.
8 Centrifuge at 13000rpm for 5 seconds.
9 Discard supernatant.
10 Add 200µl of New Wash[124].
11 Resuspend.
12 Repeat steps 8-9.
13 Add 400µl of New Wash.
14 Repeat steps 11-13.
15 Repeat steps 11-12.
16 Add 20µl of sterile water to elute.
17 Incubate at 55°C for 5 minutes.
18 Spin down at 13000rpm for at least 30 seconds.
19 Remove supernatant being very careful not to remove any pellet.
20 Add a further 5µl of sterile water to the pellet and resuspend.
21 Repeat step 20.
22 Store extracts at -70°C.

DNA EXTRACTION METHOD #2

As for method 1 but use 1.5µl of L6 lysis buffer at step 1 and 18.5µl of Sodium Iodide buffer at step 5.

DNA EXTRACTION METHOD #3

1 For mites preserved in alcohol, remove the mite from the alcohol and allow to dry thoroughly either in a vacuum drier or covered on the bench at room temperature for about five minutes.[125]
2 Freeze the mite on dry ice for about five minutes or until frozen.

---

[122] **Sodium Iodide buffer**

Geneclean II kit (BIO 101, Cat no. 3106, Stratech Scientific Ltd.). Store in the dark at +4°C.

[123] **Glassmilk**

Geneclean II kit (BIO 101, Cat no. 3106, Stratech Scientific Ltd.). Store at +4°C.

[124] **New Wash**

Geneclean II kit (BIO 101, Cat no. 3106, Stratech Scientific Ltd.). This must be diluted in ethanol and stored at -20°C and kept on ice whilst in use.

[125] It is important to remove all of the alcohol since it may inhibit subsequent PCR reactions.

3 Grind up the mite.[126]
4 When the mite is ground more or less to a powder, remove it from the dry ice and immediately add 40µl of SDS Lysis buffer [127] so that the buffer freezes in the tube.
5 Continue grinding the mite until it is homogenised in the buffer and the buffer has thawed.
6 Keeping the pestle in the tube add 10µl of 10 mg/ml Proteinase K.
7 Seal the pestle in the tube with parafilm.
8 Incubate at 37°C overnight.
9 Add 150µl 6M Sodium Iodide using it to rinse the outside of the pestle.
10 Discard the pestle.
11 Add 2µl glassmilk.
12 Incubate at room temperature for 5 minutes.
13 Centrifuge at 13000rpm for 5 seconds.
14 Discard the supernatant.
15 Repeat step 13-14 to ensure that all the NaI has been removed.
16 Add 1ml New Wash.
17 Resuspend.
18 Repeat steps 13-14.
19 Repeat steps 16-18.
20 Repeat step 19.
21 Repeat step 13 to ensure that all the New Wash has been removed.
22 Resuspend the glassmilk pellet in 35µl of sterile water.
23 Incubate at 55°C for 5 minutes.
24 Transfer the supernatant to a fresh tube.
25 Add 5µl of sterile water to the pellet.
26 Repeat step 13.
27 Repeat step 24.
28 Ensure that there is no glassmilk in the extract since very small quantities can inhibit PCR reactions.[128]
29 Store extracts at -70°C.

---

[126] If the mite thaws during this grinding process place it on dry ice to refreeze.

[127] **SDS Lysis buffer**

1 Mix 80µl of water, 100µl of 0.1M Tris HCl pH6.4 (see above) and 20µl 0.5M EDTA pH8.0 (see above).

2 Adjust the pH to 8.0

3 Add 11.688g NaCl.

4 Add 2g SDS.

5 Store at room temperature.

[128] If in doubt centrifuge the extract at 13000rpm for 2 minutes and transfer it to a fresh tube.

DNA EXTRACTION METHOD #4

1 Crush frozen mites on dry ice in a 1.5ml Eppendorf tube.
2 Add 1µl of L6 lysis buffer.
3 Incubate at 55°C for 10 minutes.
4 Add 80µl of Sodium Iodide buffer.
5 Add 2µl of Glassmilk.
6 Incubate at room temperature for 5 minutes vortexing after 2 minutes and after 4 minutes.
7 Centrifuge at 13000rpm for 5 seconds.
8 Discard supernatant.
9 Add 800µl of New Wash.
10 Resuspend the pellet thoroughly.
11 Repeat steps 7-8.
12 Repeat steps 9-11.
13 Repeat step 12.
14 Ensure that as much New Wash as possible has been removed.
15 Resuspend the pellet in 35µl of water.
16 Incubate at 55°C for 5 minutes.
17 Centrifuge at 13000rpm for 30 seconds.
18 Recover 30µl of supernatant containing DNA.
19 Add a further 10µl of water.
20 Repeat step 18.
21 Recover the remainder of the supernatant.
22 Store extracts at -70°C.

DNA EXTRACTION METHOD #5

As for method 3 but incubate at 55°C for 2 hours at step 8.

DNA EXTRACTION METHOD #6

As for method 3 but incubate at room temperature for 64 hours at step 8.

DNA EXTRACTION METHOD #7

As for method 3 but incubate at 55°C overnight with gentle shaking at step 8.

Appendix 4.2

Protocol for the Method of Primary Amplification Used in Chapter Four

1 The following are added to a sterile Eppendorf tube:
   - 10µl buffer.
   - 10µl MgCl.
   - 1µl dNTPs.
   - 2µl forward primer @ 20 picomoles/100µl.
   - 2µl reverse primer @ 20 picomoles/100µl.
   - Template DNA prepared as described above.
   - 0.5µl Taq.

2 Make up to 100µl with sterile water.

3 Add a layer of mineral oil.

4 Transfer the tubes to a Hybaid thermal cycler along with negative controls (containing everything except template DNA) and fill the empty spaces with blank tubes containing 100µl of water layered with mineral oil.

5 Run the thermal cycler as follows:
   i 94°C for 2 minutes (hot start).
   ii 92°C for 40 seconds.
   iii 45°C for 40 seconds.
   iv 72°C for 90 seconds.
   v repeat steps ii-iv 33 times.
   vi 72°C for 5 minutes.
   vii Hold at 4°C.

Appendix 4.3

Protocol for the Method for Agarose Gel Electrophoresis of Amplification Product Used in Chapter Four

1 Put 100ml of 1xTAE [129] into a sterile container.

2 Add 0.8g Seakem Agarose.

3 Add 4µl of Ethidium Bromide.

4 Heat in a microwave with the lid slightly loosened until boiling.[130]

5 Allow to cool to 'hand warm'.[131]

6 Pour the gel (about 25ml or more for thicker gels) and add the comb.[132]

7 Allow the gel at least 40 minutes to set.

8 When the gel has set remove the comb and transfer to the gel tank.

9 Pour 1xTAE into the gel tank so that it covers the gel.

10 Mix 5 volumes of primary amplification product with 1 volumes of 6x gel loading buffer.[133]

---

[129] **1xTAE**

1 Add 1 volume of 50xTAE (see below) to 49 volumes of water

2 Store at room temperature

50xTAE

1 To a container add 242g of Tris base

2 Add 57.1ml Glacial Acetic Acid

3 Add 100ml 0.5M EDTA pH8.0 (see above)

4 Make up to 1 litre with water.

5 Store at room temperature

[130] About 2 minutes and 20 seconds on full power.

[131] e.g. by running under a cold tap.

[132] Remaining gel mixture can be used again by starting at step 4 but slightly reduce the microwaving time.

[133] **6x Gel Loading Buffer**

1 To 10 volumes of water add 3 volumes of Glycerol.

2 Add 0.25% Bromophenol Blue. (Bromophenol Blue runs at about the same rate as linear double stranded DNA of 300 base pairs, Xylene Cyanol FF runs at about the same rate as double stranded DNA of 4 kilobases)

3 Add 0.25% Xylene Cyanol FF.

4 Store at 4°C.

11 Load samples onto gel.

12 Load 1 kilobase ladder onto gel consisting of size markers of various lengths so that the sizes of the primary amplification products can be estimated.

13 Run the gel at 75 volts for about 30 minutes.

14 Photograph the gel under ultraviolet light.

Appendix 4.4

Modified QIAEX II Protocol for Gel Purification of Amplification Products Used in Chapter Four

1 Excise the DNA band from the agarose gel with a clean, sharp scalpel taking as little excess agarose as possible.
2 Add the excised band to a 1.5ml Eppendorf tube.
3 Add 6 volumes of QX1 buffer[134].
4 Resuspend QIAEX II[135] by vortexing for 30 seconds.
5 Add 10µl QIAEX II.
6 Incubate at 50°C for 10 minutes vortexing every 2 minutes to keep the QIAEX II in suspension.
7 Spin at 13000 rpm for 30 seconds.
8 Add 500µl QX1 buffer.
9 Resuspend the pellet by vortexing.
10 Repeat step 7.
11 Remove all traces of supernatant.
12 Add 500µl PE[136] buffer.
13 Repeat steps 9-11.
14 Repeat step 12.
15 Repeat step 9.
16 Spin at 13000 rpm for 60 seconds.
17 Repeat step 11.
18 Air dry the pellet until it becomes white (10-15 minutes).
19 Add 20µl of water.
20 Repeat step 9.
21 Incubate at room temperature for 5 minutes.
22 Repeat step 16.
23 Transfer the supernatant to a clean tube.
24 Add 5µl of water to the pellet.
25 Repeat steps 20-22.
26 Add supernatant to that eluted in step 23.

---

[134] Catalogue number 20021. Store at room temperature.

[135] Catalogue number 20902. Store at room temperature.

[136] Add 96-100% ethanol to concentrate (catalogue number 19065) prior to use. Store at room temperature.

Appendix 4.5

Protocols for the Cleaning and Concentrating Amplification Products Used in Chapter Four

CHLOROFORM EXTRACTION

1 Add an equal volume of 24:1 $CHCl_2$: Isoamyl alcohol to the amplification reaction.
2 Mix thoroughly by vortexing.
3 Spin at 13000 rpm for 5 minutes.
4 Remove the aqueous phase and measure its volume.

MICROCON 100

1 Load the aqueous phase into the sample reservoir of a microcon-100 microconcentrator without touching the membrane.
2 Add enough TE pH8.0[137] to make the total volume in the sample reservoir up to 500µl.
3 Spin at 7000 rpm for 15 minutes.
4 Remove the bottom tube.
5 Add a further 10µl[138] of TE pH8.0[139] to the membrane.
6 Place a clean Eppendorf tube over the top of the sample reservoir.
7 Invert the entire assembly (i.e. sample reservoir plus clean Eppendorf tube).
8 Spin for 3 minutes at 4000 rpm.
9 Concentrated amplification product will now be at the bottom of the Eppendorf.
10 Store concentrated amplification product at -20°C prior to sequencing.

---

[137] **TE pH8.0**

1 Add 1.211g of Tris to a beaker

2 Add 50µl 0.2M EDTA (see above).

3 Make up to 1000ml with water.

4 Adjust to pH8.0

5 Store at room temperature.

[138] Or more if pooling the products of more than one amplification reaction i.e. 10µl of TE pH8.0 for each 100µl amplification reaction pooled.

[139] Water can also be used.

Appendix 4.6

Protocol for Manual Sequencing Used in Chapter Four

1 Add 4µl of each dideoxy mixture[140] to each of four labelled tubes.
2 Store at 4°C to avoid evaporation.
3 Dilute AmpliTaq 1:10 in the dilution buffer provided.
4 Add 3.5µl DMSO to a sterile Eppendorf tube.
5 Add 200-400ng template DNA.
6 Add 2.0µl TAQuence reaction buffer.
7 Add 2.0µl diluted AmpliTaq.
8 Add 2.0µl γ^{32}-P end labelled primer.
9 Make up to 21.0µl with millipure water.
10 Spin at 13000rpm for 30 seconds.
11 Add 4.4µl primer template mixture (steps 3-10) to each tube containing dideoxy mix (steps 1-2).
12 Repeat step 10.
13 Cover each reaction with 30µl mineral oil.
14 Transfer the tubes to a Coy thermal cycler.
15 Run the thermal cycler as follows:
   i 94°C for 30 seconds (hot start).
   ii 94°C for 30 seconds.
   iii Annealing temperature[141] for 30 seconds.
   iv 72°C for 120 seconds on the Coy.
   v repeat steps ii-iv 24 times.
16 Add 4µl of TAQuence stop mixture.

---

[140] **Di-Deoxy Mixtures**

ddGTP mix: 45µM ddGTP + 15µM each of dGTP, dATP, dTTP and dCTP (= 0.9µl ddGTP + 10.0µl 150µM dNTP stock + 89.1µl dH2O)
ddATP mix: 600µM ddATP + 15µM each of dGTP, dATP, dTTP and dCTP (= 12.0µl ddATP + 10.0µl 150µM dNTP stock + 78.0µl dH2O)
ddTTP mix: 1200µM ddTTP + 15µM each of dGTP, dATP, dTTP and dCTP (= 24.0µl ddTTP + 10.0µl 150µM dNTP stock + 66.0µl dH2O)
ddCTP mix: 450µM ddCTP + 15µM each of dGTP, dATP, dTTP and dCTP (= 9.0µl ddCTP + 10.0µl 150µM dNTP stock + 81.0µl dH2O)

150µM dNTP stock: 0.75µl of each (or 3µl mixed) dNTPs in 500ml dH_2O

[141] This depends on the primer. Unlike primary amplification sequencing was always performed at the annealing temperature derived from Sugg's formula (see above).

Appendix 4.7

Protocol for Acrilamide Gel Electrophoresis of Manual Sequencing Products Used in Chapter Four

1 To 100ml of 6% Sequencing gel[142] add 1ml 10% Ammonium Persulphate.[143]
2 Add 30µl TEMED.
3 Pour gel[144].
4 Allow to set (at least 30 minutes).
5 Set up gel.
6 Add 1xTBE[145] to upper and lower buffer tanks.
7 Flush urea crystals out of the area in which the comb will be inserted with 1xTBE using a syringe.
8 Insert comb.

---

[142] **6% Sequencing Gel**

1 Add 500g Urea to a clean beaker.

2 Add 100ml 10xTBE.

3 Add 200ml Accugel 40% (19:1) Acrylamide: Bisacrylamide Solution (from National Diagnostics).

4 Make up to 1000ml with water.

5 Store in the dark at room temperature.

10xTBE

1 To a clean beaker add 108g Tris Base.

2 Add 55g Boric Acid.

3 Add 40ml 0.5M EDTA pH8.0 (see above)

4 Make up to 1000ml with water

5 Store at room temperature

6 If crystals start to form then autoclave.

[143] **10% Ammonium Persulphate**

1 Add 1g Ammonium Persulphate to 10ml water.

2 Store at +4 for no longer than 1 week.

[144] One of the two sequencing plates should be coated with gel-slick to facilitate separation of the plates after electrophoresis and the other with Repel Silane to facilitate removal of the gel onto filter paper after separation of the plates.

[145] 1xTBE is a 1:10 dilution of 10xTBE (see above).

9 Repeat step 7
10 Load stop solution into alternate wells to check for leakage between wells.
11 Run the stop solution into the gel until the gel has warmed up.
12 Repeat step 7
13 If all is well load the samples.
14 For short runs after 30 minutes add about 70ml of 3M Sodium Acetate dissolved in 1xTBE to the bottom buffer chamber and then run for another hour. For longer runs do not add sodium acetate and run for a total of 4.5 hours.
15 Separate the two plates making sure that the entire gel adheres to only one of the plates.
16 Place the plate with the gel attached into fixative.
17 Fix for 15 minutes.
18 Place a piece of filter paper large enough to cover the entire gel over the gel.
19 Press the filter paper down onto the gel so that the gel beneath adheres to the paper.
20 Remove the filter paper with the gel attached.
21 Place the filter paper with the gel attached in a gel drier with the gel uppermost.
22 Cover the gel with a layer of cling film to prevent contamination of the gel drier with ^{32}P.
23 Dry under a vacuum for at least 45 minutes.
24 Place the gel in a photographic cassette with film and intensifying screen.
25 Store over night[146] at -70°C.
26 Develop the film.

[146] May require longer.

Appendix 5.1
Data Set for Chapter Five

REGIONS OF MISSING SEQUENCE

[1] = unreadable, [2] = not sequenced, [3] = unalignable, * = Gap

28S rDNA REGION D3-7

Amblyseius cucumeris
GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGTGTACATTGTTAGTTTCATCCAGTAAAGCGAA
TGATTAGAGGTATTGGGGCATATATAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTCTTTCTTAATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAAATGTTGAGTTAAAGTGCCTAAAGCC[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGGTCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACTATAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAGCGCGCTC
AAAGACACCAAGGGATAGGCTGGGAAGAGTTCTCTTTTCTTTTTAAGTCACTT****GTAC
CCATGGAATGGAGTAGATTTGAGATATGGGTAAGGGTGGCATAAAGCTGCTCATTTTAGAG
TAGTTCAGTTGATTCCCTTGGTCCTTGAAAATTTGAGCGTGGTGTTTATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Cornigamasus lunaris
GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTTTTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACAATGGTAGTTTCATCCAGTAAAGCGAA
TGATTAGAGGTCTTGGGGCATATGTAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTTCTCTTTCTTAATTGAAGAGCTACTAATAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAAATGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGTATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAATATGTGTTGAATGTGTATAATTAAGTATTGTCACTATAAGCGAAA
GGGAATCAGGCTAATATTCCTGAACCTACACACCGGGA[3]GTGGTAACACAAGCGTGCTC
GAAAATACCAAGTGATAGGCTGGGAAGAGTTGTCTTTTCTTTTTAAGTCATTT****GTAC
CCATGGAATGGAGTAGATTTGAGATATGGGTAGGGGTGACATAAAGCTGCTCATTTTAGAG
CAGTTCAGTTGATTTACTTGGTCCTTGAAAATTCGAGCATGGTATTTATCTTGGTGGTAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Dermanyssus gallinae
GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACATCGGTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTCTTGGGGCGTATATATCCTCAACCTATTCTCAAACTTCTAATGGGTGTGA
GTGTTCTTTCTCAATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAAATGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACTGTAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAGTGCGCTC
AAAGACACCAAGGGATAGGCTAGGAAGAGTTATCTTTTCTTTTTAAGTCACTT****GTAC
CCATGGAATGAAGTAGATTTGAGATATGGGTAAGGGTGACATAAAGCTGCTCATTTTAGAG
TAGTTTAGTTGACTCCCTTGGTCCTTGAATATTTGAGCGTGGTGTTAATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Hemipteroseius wormersleyi

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACATCTTTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTATTGGGGCGTATATAGCTTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTCTTTCTTAATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAAATGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAATCTGTGTTGAATGTGTATAATAATGTGTTATCACTGTAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAGAGCGCTC
GAAGACACCAAGGGATAGGCTAGGAAGAGTTCTCTTTTCTTTATAAGTCAATT****GTAC
CCATGGAATGGACTAGATTTGAGATATGGGTAAGGGTGACATAAAGCTGCTCATTTTAGAG
CAGTTCAGTTGATTCCCTTGGTCCGTGAATATTTGAGCGTGGTATTGATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

***Stratiolaelaps miles* [1]**

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACATCTTTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTATTGGGGCGTATATAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTCTTTCTCAATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAATTGTTGAGTTAAAGTGCCCAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACTATAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAGTGCGCTC
AAAAACACCAAGGGATAGGCTAGGAAGAGTTCTCTTTTCTTTTTAAGTCATTT****GTAC
CCATGGAATGAAGTAGATTTGAGATATGGGTAAGGGTGACATAAAGCTGCTCATATTAGAG
TAGTTTAGTTGATTCCCTTGGTCCTTGAAAATTTGAGCGTGGTGCTAATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

***Stratiolaelaps miles* [2]**

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACATCTTTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTATTGGGGCGTATATAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTCTTTCTCAATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAATTGTTGAGTTAAAGTGCCCAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACTATAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAGTGCGCTC
AAAAACACCAAGGGATAGGCTAGGAAGAGTTCTCTTTTCTTTTTAAGTCATTT****GTAC
CCATGGAATGAAGTAGATTTGAGATATGGGTAAGGGTGACATAAAGCTGCTCATATTAGAG
TAGTTTAGTTGATTCCCTTGGTCCTTGAAAATTTGAGCGTGGTGCTAATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Hypoaspis rosei

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACACCTTTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGCCTTGGGGCGTATTTAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTCTTTCTCAATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAATTGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACTATAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAGTGCGCTC
GAAAACACCAAGGGATAGGCTAGGAAGAGTTCTCTTTTCTTTTTAAGTTACTT****GTAC
CCATGGAATGAAGTAGATTTGAGATATGGGTAAGGGTGACATAAAGCTGCTCATATTAGAG
TAGTTTAGTTGATTCCCTTGGTCCTTGAAAATTTGAGCGTAGTGCTTATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Macrocheles glaber

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCATACATTTTTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTCTTGGGGCATATATAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GCGATCTTTCTCAATTGAAGAAC*GCTGCTAAATAAGTGTGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAATTGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACAATAAGCAAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAATGCGCTC
AAAGACACCAAGAGATAGGCTAGGAAGAGTTATCTTTTCTTTTTAAGTTACTT****GTGC
CCATGGAATGAAGTAGATTTGAGATATGGGTAAGGGTAACATAAAGCTGCTCATATTAGAG
TAGTTTAGTTGACTCTCTTGGTCCTTGAAAATTTGAGCGTGGTTCTAATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Pergamasus septentrionalis **[1]**

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTTTTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACAACGGTAGTTTCATCCAGTAAAGCGAA
TGACTAGAGGTATTGGGGCATATGTAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTTCTCTTTCTTAATTGAAGAGCTGCTGATAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAAATGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGTATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAATATGTGTTGAATGTGTATAATTAAGTATTGTCACTATAAGCGAAA
GGGAATCAGGCTAATATTCCTGAACCTACACACCGGGA[3]GTGGTAACACAAGTGCACTC
GAAAATACCAAGTAATAGGCTGGGAAGAGTTCTCTTTTCTTTTTAAGTTATTT****GTAC
CCATGGAATGGAGTAGATTTGAGATATGGGTAAGAGTGACATAAAGCTGCTCATTTTAGAG
CAGTTCAGTTGATTTACTTGGTCCTTGAAAATTCGAGTGTGGTAATTATCTTGGTGGTAGA
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Pergamasus septentrionalis **[2]**

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTTTTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACAACGGTAGTTTCATCCAGTAAAGCGAA
TGACTAGAGGTATTGGGGCATATGTAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTTCTCTTTCTTAATTGAAGAGCTGCTGATAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAAATGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGTATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAATATGTGTTGAATGTGTATAATTAAGTATTGTCACTATAAGCGAAA
GGGAATCAGGCTAATATTCCTGAACCTACACACCGGGA[3]GTGGTAACACAAGTGCACTC
GAAAATACCAAGTAATAGGCTGGGAAGAGTTCTCTTTTCTTTTTAAGTTATTT****GTAC
CCATGGAATGGAGTAGATTTGAGATATGGGTAAGAGTGACATAAAGCTGCTCATTTTAGAG
CAGTTCAGTTGATTTACTTGGTCCTTGAAAATTCGAGTGTGGTAATTATCTTGGTGGTAGA
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Phytoseiulus persimilis

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACATCTGTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTATTGGGGCATATATAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTCTTTCTTAATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAAATGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACTATAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAGCGCGCTC
GAAGACACCAAGGGATAGGCTGGGAAGAGTTCTCTTTTCTTTTTAAGTCACTT****GTAC
CCATGGAATGGAGTAGATTTGAGATATGGGTAAGGGTGACATAAAGCTGCTCATTTTAGAG
TAGTTCAGTTGATTCCCTTGGTCCTTGAAAATTTGAGCGTGGTGTTTATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Spinturnix myoti

ATCTGGTCAAGATGAAGCCAGAGGAAACTCTGGTGGAGGTCTGAAGTTGTTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACAAGT*TAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTATTGGGGTGTATACAGCTTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GCGTTCTTTCTTTATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAATTGTCGAGTTAAAGTGCCTAAAGCG[1]ATG
CTCATGAGATCTGTAAAGGGTGTTAGTTGCTGAAGA[2]TTGGTCGTGGGTCAGTCGGTCC
TAAGCTTGTGACGAAACATGCGGTGAATGTGTATAATGCTGTGTTATCATTGTAAGCGAAA
GGGAATCAGGCTAATATTCCTGAACCCGCGCACCGGGA[3]GTGGCAACACAAGTTTGCTC
AAAAACACCAAGGAATAGGCTGAGAAGAGTTATCTTTTCTTTTTAAGTCACTTAGCCATAC
CCATGGAATGAAGTAGATTTGAGATATGGGTAAGGGTGGCATAAAGCTGCTTGTTTTAGAG
CAGGTCAGTTGATTTTCTTGGTCCTTGAAAATTTGAGCAAAGTTCTAATCTTGGTGGCGGA
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Spinturnix plecotinus

ATCTGGTCAAGATGAAGCCAGAGGAAACTCTGGTGGAGGTCTGAAGTTGTTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACAACT*TAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTATTGGGGTGTATATAGCTTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTGTTTCTTTATTGAAGAAC*GCTGCTAAATAGGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAATTGTCGAGTTAAAGTGCCTAAAGCG[1]ATG
CTCACGAGATCTGTAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTGTGACGAAACATGCGGTGAATGTGTATAATGCTGTGTTATCATTGTAAGCGAAA
GGGAATCAGGCTAATATTCCTGAACCCGCGCACCGGGA[3]GTGGTAACACAAATTTGCTC
AAAAACACCAAGGAATAGGCTGAGAAGAGTTATCTTTTCTTTTTAAGTCACTTAGCCATAC
CCATGGAATGAAGTAAATTTGAGATATGGGTAAGGGTGGCATAAAGCTGCTTGTTTTAGAG
CAGGTCAGTTGATTTTCTTGGTCCTTGAAAATTTGTGCAAAGTTCTAATCTTGGTGGCGGC
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Typhlodromus pyri

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGCGTACATTTTTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTATTGGGGCATATATAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTCTTTCTTAATTGAAGAAC*GCTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAAATGTTGAGTTAAAGTGCCTAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGGTCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACTATAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAAGCGCGCTC
AAAGACACCAAGGGATAGGCTGGGAAGAGTTCTCTTTTCTTTTTAAGTCATTT****GTAC
CCATGGAATGGAGTAGATTTGAGATATGGGTAAGGGTGGCATAAAGCTGCTCATTTTAGAG
TAGTTCAGTTGATTCCCTTGGTCCTTGAAAATTTGAGCGCGGTGTTTATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Varroa jacobsoni

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCTAGCGTACACGTTTAGTTTCATCCCGTAAAGCGAA
TGATTAGAGGTATTGGGGCGTATATAGCCTCAACCTATTCTCAAACTTTTAATGGGTGTGA
GTGTTCTTTCTCGATTGAAGAAC*ACTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGTTGAACCAATTGTTGGGTTAAAGTGCCTAAAGCT[1]ATG
CGCATGAGATCTATAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTATGATGAAACATGTGTTGAATGTGTATAATAATGTGTTATCACTATAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGCAACACAATTACGCTC
AAAAACACCAAGGGATAGGCTAGGAAGAGTTGTCTTTTCTTTTTAAGTTACTG****GTAC
CCATGGAATGAAGTAAATTTGAGATATGGGTAAGGGTGACATAAAGCTGCTCATATTAGAG
TAGTTTAGTTGATTCCCTTGGTCCTTGAAAATTTGAGCGTGGTGATTATCTTGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCAAGGT

Veigaia nemorensis

GCCTAGTCAAGATGAAGCCAGAGGAAACTCTGGTGGATGTCTGAAGTTATTCTGACGTGCA
AATCGATAATCTGATCTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT
CCTTCCGAAGTTTCCCTCAGGATAGCAAGTGTACATTGGTAGTTTCATCCAGTAAAGCGAA
TGATTAGAGGTATTGGGGCATATGTAGTCTCAACCTATTCTCAAACTTTCAATGGGTGTGA
GTTCTCTTTCTTAATTGAAGAGTGACTGCTAAATAAGTATGCTTAGTGGGCCATTTTTGGT
AAGCAGAACTGGCGCTGTGGGATGAACCAAACGTGGGGTTAAAGTGCCTAAAGCT[1]ATG
CGCATAAGATCTACAAAGGGTGTTAGTTGCTGAAGA[2]TTGATCGTGGGTCAGTCGGTCC
TAAGCTTACGATGAAGCATATGTTGAATGTGTATAATAAAGTGT*ATCACTGTAAGCGAAA
GGGAATCAGGCTAACATTCCTGAACCTGCACACCGGGA[3]GTGGTAACACAAGTGTACTC
AAAGACACCGAGGGATAGGCTGGGAAGAGTTTTCTTTTCTTTTTAAGTTGTAT****ATTC
CCATGGAATTGAGTAGATTTGAGATATGGGCAGGCGCAACATAAAGCTACTCATTTTAGAG
TAGTTCAGTCAATTCTCTTGGTCCTTGAAAATTTGAGTACAGTAATTATCATGGTGGCAGT
CCGTACTAGTATCCGCAGCAGGTCTCCGAGGT

Appendix 5.2

Base Composition of 28S rDNA Region D3-7 Sequences with Invariant Sites Included

Species	Nucleotide N (%)			
	G	C	A	T
Outgroup				
Cornigamasus lunaris	193 (25.70)	121 (16.11)	209 (27.83)	228 (30.36)
Pergamasus septentrionalis	191 (25.43)	121 (16.11)	213 (28.36)	226 (30.09)
Outgroup Mean	**192 (25.57)**	**121 (16.11)**	**211 (28.10)**	**227 (30.23)**
Ingroup				
Amblyseius cucumeris	197 (26.27)	129 (17.20)	204 (27.20)	220 (29.33)
Dermanyssus gallinae	195 (26.00)	132 (17.60)	205 (27.33)	218 (29.07)
Stratiolaelaps miles	192 (25.60)	131 (17.47)	208 (27.73)	219 (29.20)
Hypoaspis rosei	192 (25.60)	133 (17.73)	205 (27.33)	220 (29.33)
Hemipteroseius wormersleyi	196 (26.13)	130 (17.33)	205 (27.33)	219 (29.20)
Macrocheles glaber	188 (25.07)	130 (17.33)	213 (28.40)	219 (29.20)
Phytoseiulus persimilis	196 (26.13)	131 (17.47)	204 (27.20)	219 (29.20)
Spinturnix myoti	201 (26.69)	130 (17.26)	204 (27.09)	218 (28.95)
Spinturnix plecotinus	199 (26.43)	129 (17.13)	204 (27.09)	221 (29.35)
Typhlodromus pyri	196 (26.13)	130 (17.33)	203 (27.07)	221 (29.47)
Varroa jacobsoni	193 (25.73)	128 (17.07)	208 (27.73)	221 (29.47)
Veigaia nemorensis	193 (25.73)	128 (17.07)	212 (28.27)	217 (28.93)
Ingroup Mean	**195 (26.00)**	**130 (17.33)**	**206 (27.47)**	**219 (29.20)**
TOTAL MEAN	**194 (25.87)**	**129 (17.20)**	**207 (27.60)**	**220 (29.33)**

Appendix 5.3

Base Composition of 28S rDNA Region D3-7 Sequences with Invariant Sites Removed

Species	Nucleotide N (%)			
	G	C	A	T
Outgroup				
Cornigamasus lunaris	31 (22.63)	17 (12.41)	37 (27.01)	52 (37.96)
Pergamasus septentrionalis	29 (21.17)	17 (12.41)	41 (29.93)	50 (36.50)
Outgroup Mean	**30 (21.90)**	**17 (12.41)**	**39 (28.47)**	**51 (37.23)**
Ingroup				
Amblyseius cucumeris	35 (25.74)	25 (18.38)	32 (23.53)	44 (32.35)
Dermanyssus gallinae	33 (24.26)	28 (20.59)	33 (24.26)	42 (30.88)
Stratiolaelaps miles	30 (22.06)	27 (19.85)	36 (26.47)	43 (31.62)
Hypoaspis rosei	30 (22.06)	29 (21.32)	33 (24.26)	44 (32.35)
Hemipteroseius wormersleyi	34 (25.00)	26 (19.12)	33 (24.26)	43 (31.62)
Macrocheles glaber	26 (19.12)	26 (19.12)	41 (30.15)	43 (31.62)
Phytoseiulus persimilis	34 (25.00)	27 (19.85)	32 (23.53)	43 (31.62)
Spinturnix myoti	39 (28.06)	26 (18.71)	32 (23.02)	42 (30.22)
Spinturnix plecotinus	37 (26.62)	25 (17.99)	32 (23.02)	45 (32.37)
Typhlodromus pyri	34 (25.00)	26 (19.12)	31 (22.79)	45 (33.09)
Varroa jacobsoni	31 (22.79)	24 (17.65)	36 (26.47)	45 (33.09)
Veigaia nemorensis	31 (22.79)	24 (17.65)	40 (29.41)	41 (30.15)
Ingroup Mean	**33 (24.26)**	**26 (19.12)**	**34 (25.00)**	**43 (31.62)**
TOTAL MEAN	**32 (23.53)**	**25 (18.38)**	**35 (25.74)**	**44 (32.35)**

Appendix 5.4

AT Biases of 28S rDNA Region D3-7 Sequences with Invariant Sites Included

Species	Total Number of Nucleotides	AT Bias	GC Skew	AT Skew
Outgroup				
Cornigamasus lunaris	751	0.0155	0.2293	-0.0435
Pergamasus septentrionalis	751	0.0155	0.2244	-0.0296
Outgroup mean	-	**0.0155**	**0.2269**	**-0.0366**
Ingroup				
Amblyseius cucumeris	750	0.0115	0.2086	-0.0377
Dermanyssus gallinae	750	0.0104	0.1927	-0.0307
Stratiolaelaps miles	750	0.0110	0.1889	-0.0258
Hypoaspis rosei	750	0.0103	0.1815	-0.0353
Hemipteroseius wormersleyi	750	0.0111	0.2025	-0.0330
Macrocheles glaber	750	0.0117	0.1824	-0.0139
Phytoseiulus persimilis	750	0.0107	0.1988	-0.0355
Spinturnix myoti	753	0.0110	0.2145	-0.0332
Spinturnix plecotinus	753	0.0116	0.2134	-0.0400
Typhlodromus pyri	750	0.0112	0.2025	-0.0425
Varroa jacobsoni	750	0.0121	0.2025	-0.0303
Veigaia nemorensis	750	0.0119	0.2025	-0.0117
Ingroup mean	-	**0.0112**	**0.1992**	**-0.0308**
TOTAL MEAN	-	**0.0118**	**0.2032**	**-0.0316**

Appendix 5.5

AT Biases of 28S rDNA Region D3-7 Sequences with Invariant Sites Removed

Species	Total Number of Nucleotides	AT Bias	GC Skew	AT Skew
Outgroup				
Cornigamasus lunaris	137	0.0448	0.2917	-0.1685
Pergamasus septentrionalis	137	0.0440	0.2609	-0.0989
Outgroup mean	-	**0.0444**	**0.2763**	**-0.1337**
Ingroup				
Amblyseius cucumeris	136	0.0134	0.1667	-0.1579
Dermanyssus gallinae	136	0.0074	0.0820	-0.1200
Stratiolaelaps miles	136	0.0108	0.0526	-0.0886
Hypoaspis rosei	136	0.0102	0.0169	-0.1429
Hemipteroseius wormersleyi	136	0.0105	0.1333	-0.1316
Macrocheles glaber	136	0.0186	0.0000	-0.0238
Phytoseiulus persimilis	136	0.0097	0.1148	-0.1467
Spinturnix myoti	139	0.0107	0.2000	-0.1351
Spinturnix plecotinus	139	0.0147	0.1935	-0.1688
Typhlodromus pyri	136	0.0140	0.1333	-0.1842
Varroa jacobsoni	136	0.0169	0.1273	-0.1111
Veigaia nemorensis	136	0.0140	0.1273	-0.0123
Ingroup Mean	-	**0.0126**	**0.1123**	**-0.1185**
TOTAL MEAN	-	**0.0171**	**0.1357**	**-0.1207**

Appendix 5.6

A Note on Male Reproductive Characters in the Mesostigmata

SPERM TRANSFER

In the cohort Dermanyssina the male genital orifice is located in a presternal position and the movable digit of each chelicera is provided with a **spermatodactyl** (also called the **spermatophoral process** or the **spermatostyle**), a free appendage arising from the basal region of the movable digit usually provided with a sperm transfer tube (Young (1959) cited in Evans (1992))[147]. The male uses the spermatodactyl to transfer the sperm to the copulatory pores of the female[148] which in the Dermanyssina are on or around the base of the legs (see appendix 7.1) for which reason this method of sperm transfer is known as **podospermy**.[149] In all other cohorts of the Mesostigmata except the Parasitina the genital orifice of the male is usually located within the sternogenital shield, typically in the region of Coxae II and III with no modification of the chelicerae for sperm transfer.[150] In these groups sperm are transferred directly to the female genital opening (**gonopore**), a method of sperm transfer which is known as **tocospermy**. The Parasitina exhibit characteristics intermediate between those of tocospermic and podospermic forms. Females are tocospermic with respect to the site of introduction of the spermatophore, however, males resemble the podosperms of the Dermanyssina with respect to the position of the genital opening and modification of the movable digit of the chelicerae into a structure known as a **spermatotreme**, a foramen midway along the movable digit through which the neck of the spermatophore passes during transfer to the female.[151]

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[147] The shape of the spermatodactyl is invariably species specific. In some parasitic Dermanyssoidea the spermatodactyl is fused with the greater part of the digit but remains free distally.

[148] During insemination the tip of the spermatodactyl is introduced through the solenostome into the infundibulum (Amano and Chant, 1978). (See figure A7.1.1.1 in appendix 7.1.)

[149] Since podospermy is a synapomorphic character for the Dermanyssina it may help to explain the extraordinary success of this group in terms of the phenomenal diversity of the ecological niches into which they have radiated as well as simply the large number of species. Podospermy may allow longer storage of sperm, for example, which will decrease he need for a female to find subsequent mates (and incur the costs of having to do so) once she has mated once. In arrhenotokous species the ability to store sperm can be translated into the ability to control the sex ration very precisely over a long course of time by selective fertilisation of the eggs (see Chapter 1). Alberti and Hanel (1986) consider podospermy to be a more efficient method of sperm transfer than tocospermy therefore fewer spermatozoa are introduced into the female in podosperms (30-40 in *Varroa*) than in tocosperms (Faasch, 1967), and they suggest that this may provide the key to understanding the species richness of this group.

[150] Exceptions are *Liroaspis togatus* (Sejina), *Celaenopsis badius* (Antennophorina) and *Fuscuropoda hilli* (Uropodina) in which the genital orifice is in the presternal position (Evans, 1992).

[151] The sperm access systems of the Dermanyssina and Parasitina are referred to collectively as **neospermy** and those of the remainder of the Mesostigmata and the ticks as **archispermy** (Alberti, 1988).

SPERM MORPHOLOGY

All of the Acari have sperm of the **aflagellate type** and motility has been acquired secondarily by means of new organelles (Baccetti, 1979). There are two types of sperm found in the Mesostigmata; the **vacuolated type** and the **ribbon type** (Alberti, 1984; Alberti, 1991).

The Vacuolated Type of Spermatozoa

The Uropodina, Epicriina and Zerconina share with the ticks the presumably primitive vacuolated type of spermatozoa (Alberti, 1980). In the course of spermatogenesis a large vacuole, lined with cytoplasmic processes, forms through the fusion of golgi derived vesicles ((Breucker and Horstmann, 1972; Feldman-Musham and Filshie, 1979; Oliver and Brinton, 1973; Wuest *et al.*, 1978). The final stage of spermatogenesis occurs in the female genital tract and is referred to as **capacitation** or **spermateleosis**. Certain secretions of the accessory gland initiate the opening of the operculum of the cell at its anterior pole and the eversion of a cytoplasmic column (previously protruding into the vacuole from the posterior pole) through it, turning the entire cell inside out so that the wall of the vacuole, with its processes, forms the outer wall of the capacitated spermatozoan. This process is coupled with a doubling in length of the cell (Baccetti, 1979)

The Ribbon Type of Spermatozoa

Characteristic features of the ribbon type of sperm shared by the Parasitina and Dermanyssina include absence of a large vacuole and presence of longitudinally directed ribbon like structures or stiff bands derived from sac-like invaginations (flat chambers) (Witalinski, 1975; Witalinski, 1979) assumed to homologous to the large vacuole, or its smaller precursors, of the vacuolated spermatozoa (Alberti and Hanel, 1986). Ribbon sperm are not turned inside out during capacitation which occurs in the rami (see figure A7.1.1 in appendix 7.1) by a modification of shape leading to elongation. In *Varroa jacobsoni* the nucleus elongates and the chromatin changes structure whilst in the cytoplasm filamentous structures become visible These filamentous structures are considered to enable the sperm cells to arrange themselves in one direction in the receptaculum (see appendix 7.1). Ribbon sperm have only been found in those Mesostigmata in which the male genital orifice is presternal and the chelicerae are modified as gonopods. In the final stage of spermatogenesis, in *Varroa jacobsoni* at least, the spermatozoa have become elongate fusiform bodies and lost their ribbons of flat chambers which are thought to be transferred to the cell surface and integrated.

Appendix 6.1
Data Sets for Chapter Six

MOLECULAR CHARACTER STATES

0 = A, 1 = C, 2 = G, 3 = T, 4 = GAP

MORPHOLOGICAL CHARACTER STATES

= character states 1 and 2

MORPHOLOGICAL DATA

Dermanyssus gallinae
01131100001111?000110002313002000212233111100?01011001?1000001
11131?101?32121022520002110000001001000110011111011101011100 0
1032102001101101111100?0000001

Haemogamasus pontiger
01031120101150000011200011200200001023211100?01031001?1102002
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??32112021??1101101100?0000000

Hypoaspis blattae
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01020012011012131012001000201000000000110001011110001110000000
00321020210011011011 00?0000000

Macrocheles muscadomesticae
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0002012200#012531212111000101110000110111111111111100110111011
00321223310011011011 01?0000000

Parasitus coleoptratorum
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0132121??0??1101101100?0000000

Pergamasus runcatellus
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0132231??0??1101101100?0000000

Stratiolaelaps miles
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?02213202110110110110 0?0000000

Typhlodromus pyri
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011202120222111012010010100010001011110010111101011101011110?
???213203100110110110O?0000000

Veigaia nemorensis
2??3011011115030000120001021100000110111100102103012?01?010001
01021?120330105212323112132010000000001100010010100011100000O
0???012031101100100100?0000000

MOLECULAR DATA

Cornigamasus lunaris
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320330202231332222103032302113100113033131000133330032223232O
233131333133003320020213013003000300230321330232221103333322 3
002102001322121323222332001100032332023300023211300021303212 3
032020313030002223233023321320020332031232222310231223113002 13
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202302033320203032223022223201030002132131033330202102331023 3
203330133223113320000331202103223033303133223223023112301302 3
031121021022313110022 3

Dermanyssus gallinae
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113311200233311131022030210021230103122302333103111230002120O
320330202231332222123030303113100113033131000133130032223232O
232331333131003320020014213213000300230321330232221103333322 3
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032020313030002223233023321320020332031232222310231223113002 13
303203200010323233200323230300300323233031013230021200022200 3
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002220302213022002023303133331333330023101334442301110322003 2
002302033320203032223002223201030002132131033330202302333023 3
201311133223113320030333202123223233003133223221023112301302 3
031121021022313110022 3

Hypoaspis rosei
211302310020320021102022000131322322032313200233033132012321O
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320330202211332222123033302113100113033131000133330032223232O
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002302033320203032223002223201030002132131030330202302333023 3
203311133223113320000333202123023213303133223221023112301302 3
031121021022313110022 3

Macrocheles glaber

2113023100203200211020220001313223220323132002330331320123210
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1133112002333111310220302100210301033333023331031112300021200
3203302022313322221030303021131001130331310001333300322232320
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0023020333202030322300222300103000213213103033020230233302 33
2013131332231133200003332021232233130031332232210231123013023
0311210210223131100223

Pergamasus septentrionalis

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1133112002333111310220302100212301001223023331031102300021200
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2023020333202030322300202320103000213213103333020210233102 33
2033301332231133200003312023232230033031332232302011230130 23
0311210210223131100223

Stratiolaelaps miles

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0023020333202030322300222320103000213213103033020230233302 33
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0311210210223131100223

Typhlodromus pyri

2113023100203200211020220001313223220323132002330331320123210
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2033111332231133200003332021212232333031332232210231123013023
0311210210223131100223

Varroa jacobsoni

211302310020320021102022000131322322032313200233033132012321
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031121021022313110022 3

Veigaia nemorensis

211302310020320021102022000131322322032313200233033132012321
003120300313203132223030222212000201300312001103130230213223
311331120023331113102203021002323010332230233310311023000212
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COMBINED DATA

Dermanyssus gallinae

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232330031332232210231123013023031121021022313110022 3

[*Haemogamasus* + *Varroa*]

010311201011500000112000112002000010232111 00?01031001?1102002
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3333002330132444230111032200320023000333202030322230022232010
3000213213103033020230233302332033111332231133200003332021232
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Hypoaspis

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Macrocheles

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PARASITINAE

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PERGAMASINAE

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333300233033344423011103220032202302033320203032223002023201 0
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230033031332232302011230130230311210210223131100223

PHYTOSEIIDAE

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???213203100110110110 0?000000211302310020320021102022000131 3
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002013003120011031302302132233113311200233311131022030210021 2
301033333023331031112300021200320330202230332222103030302113 1
001130331310001333300322232320232331333133003320020014213213 0
003002303213302322211033333223002102001322121323222332001100 0
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003232330310130300212000222003102213001033113200113210101122 2
023221001010021212131000201011002220302213222002023313133331 3
333300231033344423011103220032202302033320203032223002223221 0
300021321310333302023023310233203311133223113320000331202123 2
232333031332232210231123013023031121021022313110022 3

Stratiolaelaps miles

012301110011202000112010 1?2112000011030111 00?00211001?1111001
010200120110122312120012002010000000001100010111100011100 00??
?022132021101101101 00?000000211302310020320021102022000131 3
223220323132002330331320123210003120300313203132223030222212 0
002013003120011031302302132233113311200233311131022030210021 2
301031333023331031112300021200320330202230332222123030302113 1
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003002303213302322211033333223002102001322121323222332001100 3
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003232330310130300212000222003102213001033113200113210101122 2
023221001010023212131000001011002220302213022002023313133331 3
333300231033344423011103220032002302033320203032223002223201 0
300021321310303302023023330233203311133223113320000331202123 2
232130031332232210231123013023031121021022313110022 3

VEIGAIIDAE

2??301101111503000012000102110000011011110010210301?01?010001
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0???012031101100100100?000000021130231002032002110202200013 13
223220323132002330331320123210003120300313203132223030222212 0
002013003120011031302302132233113311200233311131022030210023 2
301033223023331031102300021200320330202230332222103032302313 1
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Appendix 7.1

A Note on the Sperm Access Systems of the Dermanyssina

PODOSPERMY

In the Dermanyssina males pass sperm directly to a pair of pores called the **solenostomes** situated in the arthroidal membrane of the female connecting the coxae of legs III to their acetabula (Michael, 1892) or (in the Rhodacaroidea in which there is considerable variation in the position of the solenostomes) into or near the acetabula of legs IV, on the metapodal shield posterior to coxae IV or on the trochanters and femora of legs III (Athias-Henriot, 1968; Evans and Till, 1979; Lee, 1974). This association of the copulatory pores with the legs is referred to as **podospermy** (as opposed to **tocospermy** in which sperm is transferred directly to the female genital opening (**gonopore**) which is the rule in all other cohorts of the Mesostigmata). Two main forms of podospermy are present, the **laelapid type** which is found in the Veigaioidea, Eviphidoidea, Rhodacaroidea, Dermanyssoidea and some families of Ascoidea (Halolaelapidae, Ameroseiidae, Antennoseiidae, Podocinidae and some Ascidae), and the **phytoseiid type** which is only found in the Ascoid families Phytoseiidae, Otopheidomenidae and some Ascidae.

The Laelapid Type of Sperm Access System

In the laelapid type of sperm access system (figure A7.1.1) each solenostome leads, via a flat chamber or **infundibulum**, to a cuticle lined duct called the **tubulus annulatus** (also called the **canal adductur** or **sperm access duct**). This in turn leads, via a **cuticular valve**, into a wider tubular structure, the **ramus** (which, in *Varroa jacobsoni* at least, is probably a syncitium since Albert and Hanel (1986) failed to detect cell boundaries in the epithelium. The two rami join and usually enlarge to form the bladder-like **sacculus foemineus** or **'poche de maturation'**(Fain, 1963)[152]. An unpaired median duct, the **sperm duct** (or **seminal egress duct**) leads from the sacculus or fused rami. Histologically this is a continuation of the rami/sacculus. This duct, which may be enlarged to form a **sperm reservoir,** connects to a large sac-like organ, the **spermatheca** or **receptaculum seminis** (Akimov and Yastrebtsov, 1984) to avoid confusion with the use of the term spermatheca for the entire sperm access system. The combined sperm duct and receptaculum seminis is also referred to as the **cornu sacculi**. The method by which sperm reaches the ovary is an open question. Akimov and Yestrebtsov (1984)

[152] Although this does not occur in *Varroa jacobsoni* (Alberti and Hanel, 1986).

consider that the spermatozoa in *Varroa jacobsoni* penetrate the wall between the receptaculum

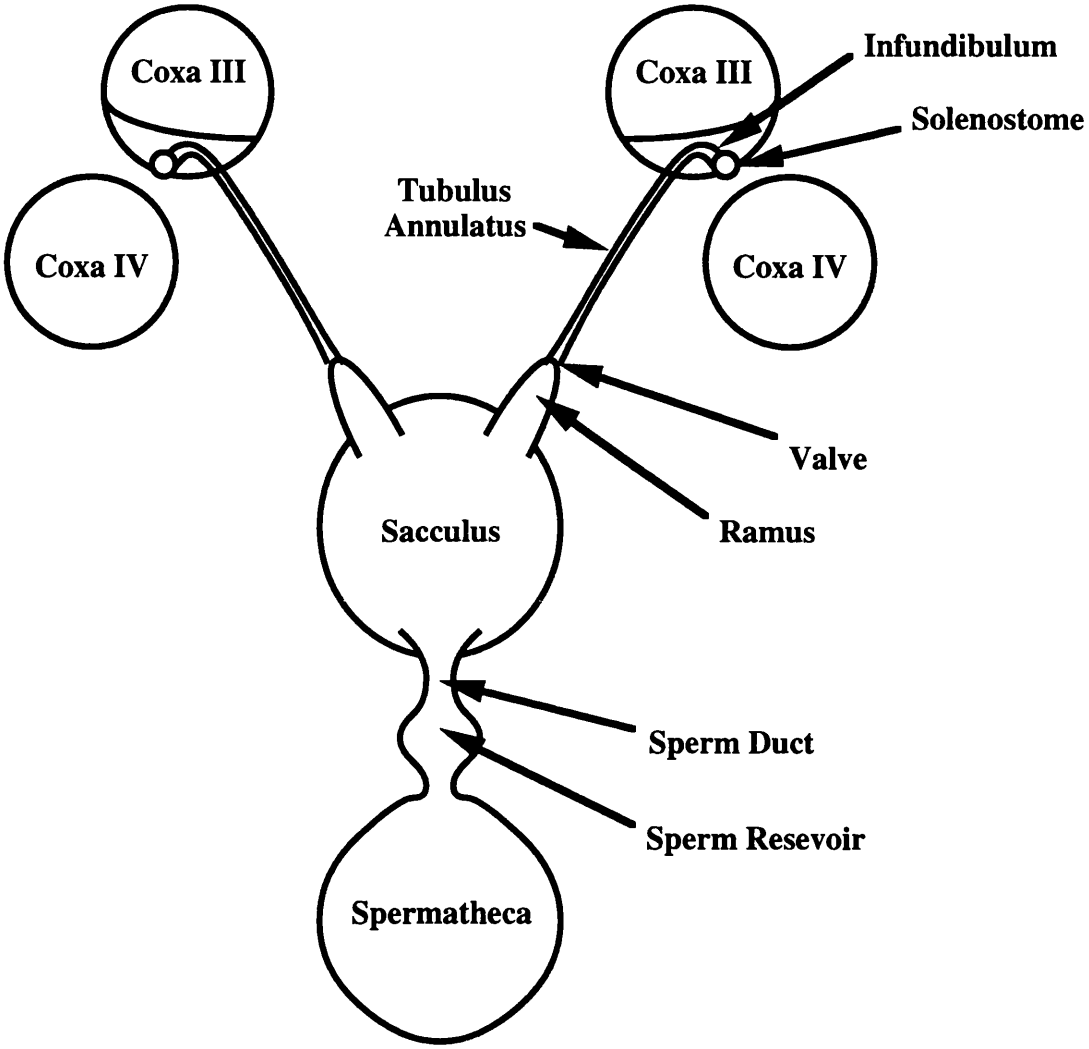

Figure A7.1.1: A Diagrammatic Representation of The Laelapid Type of Sperm Access System

After Evans (1992), figure 9.15A, p.286

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and the ovary. Alberti and Hanel (1986) disagree. They think that sperm reach the ovary by invagination into the **camera spermatis**.

The Phytoseiid Type of Sperm Access System

The phytoseiid type of sperm access system consists of discrete paired structures leading from each solenostome (figure A7.1.2). These are important taxonomic characters in the Phytoseiidae since they resist maceration during preparation for microscopic study. Homologies between the components of the two systems have so far proved impossible. It is unknown how sperm get from the vesicle to the ovary where fertilisation is assumed to take place.

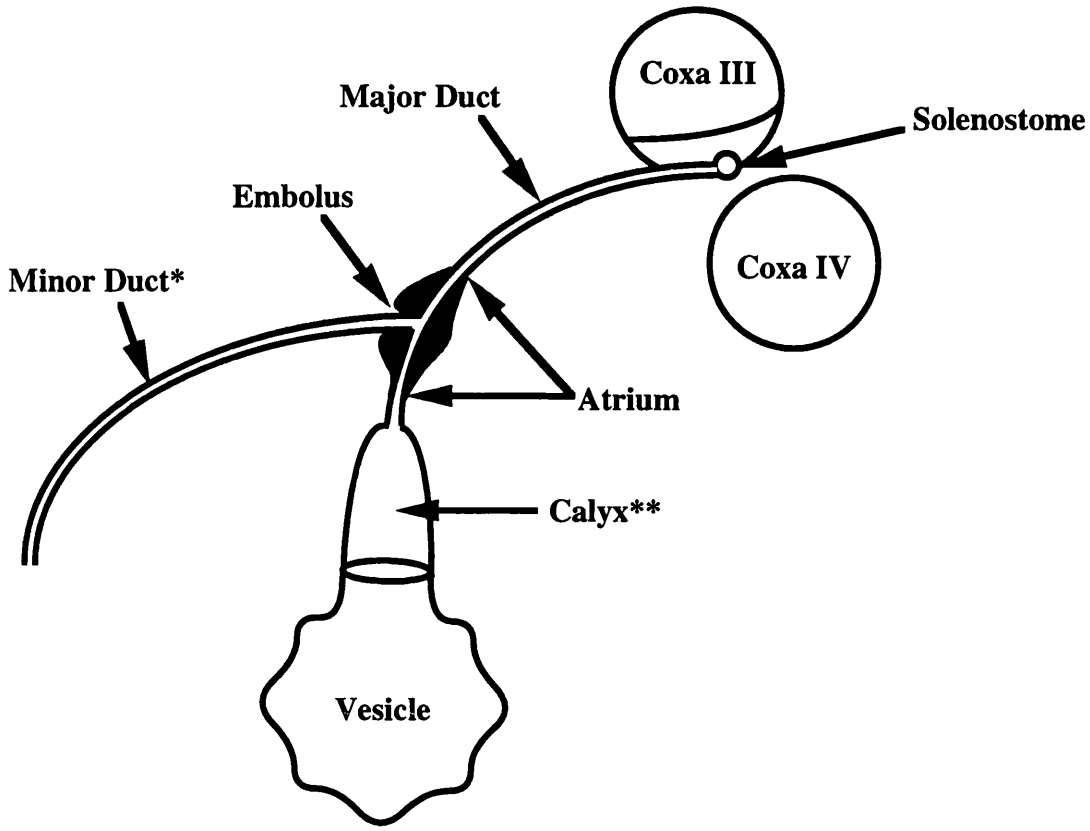

Figure A7.1.2: A Diagrammatic Representation of The Phytoseiid Type of Sperm Access System

After Evans (1992), figure 9.15B, p.286

* The **minor duct** may be a second "sperm duct" although its narrow diameter is unlikely to allow the passage of spermatozoa without considerable distension.

** In North America the **calyx** is known as the **cervix**. In Europe, however, the term cervix is used to denote the connection between the calyx and the atrium.

EVOLUTION OF PODOSPERMY

Alberti and Hanel (1986) have hypothesised that podospermy evolved from tocospermy via an intermediate in which spermatozoa penetrated the integument into the haemolymphatic space and reached the ovary by active migration. An analogous method of sperm transfer occurs in the onycophoran *Opisthopatus cintipes* in which penetration of the integument is facilitated by use of the pores of cuticular glands (Storch and Ruhberg, 1977). Formation of access ducts (tubuli, rami, and sperm ducts), they suggest, evolved later, either by deepening of the copulatory pore or by adaptation of existing cuticular gland ducts.

EVIDENCE FOR AN EXCRETORY FUNCTION FOR THE SPERM ACCESS SYSTEM

The sacculus and rami of *Haemogamasus* are capable of endosmosis and exosmosis (Michael, 1892). Females recently fed to repletion on heparinised blood excrete droplets of liquid from their solenostomes and blue dye injected into the haemocoele is taken up by the sacculus and rami and stains the liquid excreted from the solenostome (Young (1959) cited in Evans (1992)). These observations suggest that the sperm access system may have an additional role in excretion.

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